13.

The Life History and Bionomics of the Trematode, *Zygocotyle lunata* (Paramphistomidae).

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(Plates I–IV).

Zygocotyle lunata belongs in the family Paramphistomidae Fischoeder, 1901. Diesing (1836) described the species from material collected by Natterer, in Brazil, from the ceca of several species of water birds and from the cecum of the deer, *Cervus dichotomus*, and named it *Amphistoma lunatum*. Fischoeder (1903) redescribed the species from 4 whole mounts of Diesing's original material in the Vienna Museum. Stunkard (1917) erected the genus *Zygocotyle*, to contain A. *lunatum* and a new species, *Z. ceratosa*, described by him from the intestine of a duck, *Anas platyrhynchos*, from Nebraska. Price (1928) showed that *Z. ceratosa* was specifically identical with *Z. lunata*.

The host relationships of the genus *Zygocotyle* are of considerable interest. Diesing (1836) described it from the cecum of a deer, *Cervus dichotomus*, and also from the ceca of the birds, *Anas melanotus*, *A. epecutiri* and *Himantopus wilsonii*. Fischoeder (1903), after an examination of 4 of Diesing's specimens, concluded that the record of the ruminant *Cervus dichotomus* as a host of this form was probably due to an error in labelling. As pointed out by Price (1928), Dujardin (1845) and Diesing (1850) had earlier arrived at the same conclusion because of the wide difference in hosts. The suspicion of error persisted until the occurrence of *Zygocotyle* in both birds and mammals was definitely shown by Price (1928) who studied specimens of an amphistome reported by Hall (1927) from the cecum of a cow, *Bos taurus*, and identified them as *Zygocotyle lunata*, thus confirming and validating Diesing's original record. In the present study, infections with *Z. lunata* have been experimentally produced in the sheep, *Ovis aries*, and in the rat, *Mus norvegicus*, as well as in ducks. Thus a rodent as well as another ruminant is here added to the list of mammalian hosts. All the rats exposed (more than 60) became infected.

Several years ago the writer (1930, 1936) described a new species of cercaria, *C. poconensis*, from *Helisoma antrosum* collected near Henryville, Pennsylvania. Life history studies with the material in an attempt to identify the adult stage failed, and the investigation had to be abandoned because the infestation was no longer present in the snails from that region. In September, 1937, one specimen of *Helisoma antrosum* from Prospect Park Lake in New York showed an infestation with *Cercaria poconensis*, and subsequent collection of 450 snails from the same lake during October yielded 3 additional infections. Cercariae from these 4 naturally infested snails encysted in dishes in the laboratory and furnished the material for feeding experiments. Metacercariae fed to rats, ducks and sheep developed into adults of *Zygocotyle lunata* in the ceca of these hosts. Eggs, collected from feces, developed in the laboratory and young laboratory-raised snails of the species *Helisoma antrosum*, on exposure to the hatched miracidia, became infected, yielding the sporocyst, redial and cercarial stages, thus demonstrating experimentally the complete life cycle. Preliminary reports were presented in abstract form (Willey, 1937, 1938).

Gower (1938) in a study of trematodes infesting wild ducks in Michigan obtained an experimental infestation in a half-grown mallard duck of 46 immature specimens of *Zygocotyle lunata* with metacercariae from a naturally infected snail identified as *Helisoma trivolvis*.

Much progress has been made in recent years in the demonstration of North American trematode life cycles. Most of the advances have been made, however, in groups other than the amphistomes. Life cycle studies in this group, with the exception of that of Bennett (1936) on *Cotylophoron cotylophorum*, are mostly incomplete, since they lack one or more stages or fail to provide experimental proof in support of conclusions based on morphological similarity.

The demonstration of a complete life cycle requires that all stages be described and obtained experimentally. The studies may begin with any step in the sequence. In trematode studies either of two modes of attack are commonly employed. One may start with the cercarial stage,

obtained from naturally infected snails, and expose various possible hosts to it or to the metacercaria, if the cercaria encysts in the open. Such experimental hosts must be known to be free from previous infection which might be confused with the experimental one. Frequently, the structure and activities of the cercariae together with information on the fauna occurring in the same environment will give some indication of the next host. Similarly, one might start with the adult stage of the trematode in the final host. Eggs are then collected and permitted to develop and various species of snails are then exposed to the hatched miracidia. Successful infestation provides the asexual stages in the snail. In either method, the cycle is followed back to the starting point.

In the present study the investigation began with 4 naturally infected snails and, as described later, infection was obtained in the final hosts. The rats, all of which became infected, had been laboratory-raised for many generations and the ducks were known to be previously uninfected, because none in a control series of ducks, being used for other experiments, ever showed any eggs or specimens of *Zygocotyle lunata*, while all the experimentally-fed ducks became infected with the species. The complete life cycle was passed through several times in the laboratory. No further collections of naturally infected snails were made since ample material was available in the laboratory throughout the investigation. The life cycle as here presented begins with a description of the eggs obtained from experimentally infected hosts, after which the succeeding generations are considered in the order of their occurrence. The materials and methods employed with the various stages are described separately for each stage.

Egg.

The eggs of *Zygocotyle lunata* examined alive from feces or from living unstained worms are colorless. The shell is very delicate and as studied in optical sections under high magnification, it does not exceed 1.5 μ in thickness. If pushed about with a blunt needle the eggs are easily dented and burst readily with rough handling (Fig. 1). They are broadly ovoid and show little variation in shape (Fig. 2). The slightly attenuated end bears an operculum measuring from 28 μ to 31 μ in diameter. The edges of the operculum are irregularly notched and these notches interdigitate closely with similar irregular notches on the edge of the shell. A slight excrescence, from 10 to 15 μ in diameter, occurs on the shells of all the eggs at the broad end. The freshly deposited egg contains a large number of granular yolk masses suspended in a transparent fluid; and the ovum, measuring from 20 μ to 25 μ in diameter, still unsegmented, lies embedded between the vitelline masses in the opercular half of the egg (Fig. 1).

Eggs of *Z. lunata* vary much in size. Several hundred eggs collected from feces of ducks and rats infected with newly matured worms, as well as eggs from infestations of more than a year's duration, were measured. Eggs from feces of a duck and of a rat, each of which had been infested from a single feeding of metacercariae 13 months previously, show no significant difference in size. The eggs from the duck varied in length from 132 μ to 152 μ and those from the rat from 132 μ to 158 μ. Width varied in eggs of the duck parasites from 92 μ to 102 μ and in those from the rat from 89 μ to 102 μ. The average size of 38 eggs deposited by worms in the duck was 142 μ by 98 μ, while that of the 38 eggs from the rat feces measured 141 μ by 96 μ. The variation in size of eggs from these 13-month-old worms was 26 μ in length and 13 μ in width. Bennett (1936) showed a similar size range (30 μ in length and 10 μ in width) in eggs of *Cotylophoron cotylophorum*.

Fifty-six eggs of young, newly mature *Z. lunata* collected in a fecal sample from a duck experimentally infected 48 days before, varied in length from 132 μ to 152 μ and in width from 99 μ to 102 μ, the average measurements for the group being 145 μ by 99.5 μ. Similarly, 54 eggs of young, newly mature worms collected in a fecal sample from a rat infected 49 days before, varied in length from 132 μ to 158 μ and in width from 92 μ to 105 μ, the average measurements for this group being 141 μ by 98.8 μ. As with eggs from older infestations, no significant difference in size appears between eggs of worms in the 2 hosts. Further, it may be seen that the size range is the same for eggs from new and old infestations. While in the eggs measured in these series, the average length and width of eggs from young infestations slightly exceed those of older infestations, the difference is not significant.

Thirty eggs measured in stained whole mounts of newly matured worms varied from 132 by 86 μ to 152 by 99 μ, the average being 143 by 91 μ. While the average width (91 μ) of these eggs in stained and mounted young specimens is about 5 μ less than that of living eggs from older worms, the extremes in both dimensions are practically the same in eggs from fixed and stained specimens of all ages as in eggs from feces. The average obtained for all eggs of *Z. lunata* measured in the present investigation is 142 μ in length and 96 μ in width.

Price (1928) tabulated measurements of *Z. lunata* from different hosts and showed a remarkably wide variation in egg size. The eggs measured by Price and others from 7 host species vary from 124 to 153 μ in length and from 72 to 96 μ in width, the extreme range of variation being 29 μ in length and 24 μ in width. This range is somewhat wider than in the present study. All of the material used by the present author for egg measurements was obtained from experimental infestations with metacercariae from 2 naturally infected snails collected from the same small pond. The hosts yielding the specimens of *Z. lunata* for egg measurements as tabulated by Price were natural infections studied under different conditions by different workers and collected from widely separated parts of

North and South America. The differences in egg size may possibly be explained by this difference in source of material. With regard to egg size in amphistomes, it is difficult to draw any conclusion of value other than that egg size is of little importance in classification within a family.

Miracidium.

Development. Studies on development of the miracidium of *Zygocotyle lunata* were carried out on eggs obtained from feces of experimentally infected ducks and rats. All eggs observed were in the one-cell stage at the time of deposition. Much difficulty, due to bacteria and molds, was experienced in obtaining successful development of the ovum. The best results were obtained by keeping the eggs in petri dishes and changing the water twice daily. Immediately after collection from feces, the eggs were washed through 8 or 10 changes of tap water. Other methods involving (1) the use of sterile water, (2) gentle mechanical agitation and (3) a method by which drops of water fell continuously into dishes of water containing eggs were all found to be unsatisfactory. Eggs allowed to develop at a constant temperature of 25° C. in an incubator gave somewhat better results than those exposed to varying temperatures in the laboratory. By transferring the eggs twice daily with a micropipette to dishes of clean water, about 50 to 60 per cent of the embryos hatched and even then it was necessary to clean the mold from some of them with dissecting needles before they would hatch. In no case was it possible to obtain complete development without some mold forming on the eggs.

Bennett (1936) described in considerable detail the developmental stages of the miracidia of *Cotylophoron cotylophorum* and found a remarkable similarity in the sequence of organ development between that species and development in other species of trematodes as described by Thomas (1883), Looss (1892, 1896), Ortmann (1908), Johnson (1920), Stunkard (1923), Barlow (1925), Ishii (1934) and Suzuki (1931). Bennett (1936) says, "The development of the miracidium as described here coincides in practically every detail with the development of the miracidia described by the workers mentioned earlier in this discussion. This result points to the conclusion that the chronological sequence of organ development in trematode miracidia is essentially the same."

In view of Bennett's complete account of development in *C. cotylophorum*, a closely related amphistome, and the similarity manifested in the developmental history of miracidia in trematodes generally, detailed observations on the sequence of organ development are not recorded here for *Zygocotyle lunata*. However, all stages were observed in the course of the present study, using living material in hanging drops, and the process in *Z. lunata* follows very closely that described for *C. cotylophorum*.

The time required for development and hatching varies, the minimum time being 19 days and the maximum 40 days in the material of *Z. lunata* studied under laboratory conditions. Between these extremes the rate of development varied with the season of the year, more time being required during the colder than in the warmer months. Most of the eggs were embryonated at room temperature, and during the winter the room was much cooler during both day and night than in the summer. Temperature is probably the factor controlling speed of development, but controlled experiments using different temperatures at the same season of the year were not conducted. Table 1 shows the relation between time required for development and season of the year. In each case the time noted is that time elapsed from deposition of the eggs until emergence of the first miracidium in the given batch of eggs. In all the cultures, hatching was spread out over periods of 4 days or longer, indicating individual differences either in maturity of the eggs at deposition or in developmental rate. In a culture of developing miricidia of *Z. lunata*, most of the embryos are at about the same stage of organ formation but some develop more slowly than others in the same dish.

Table 1.

Seasonal variation in developmental rate of miracidia of *Zygocotyle lunata*.

Date of deposition of eggs	Date of hatching of first miracidia	Days elapsed
Dec. 2, 1937	Jan. 10, 1938	39
Dec. 4, 1937	Jan. 13, 1938	40
Dec. 16, 1937	Jan. 18, 1938	33
Jan. 22, 1938	Feb. 16, 1938	25
Feb. 2, 1938	Feb. 27, 1938	25
Feb. 23, 1938	March 21, 1938	26
March 5, 1938	March 27, 1938	22
March 14, 1938	April 7, 1938	24
April 15, 1938	May 8, 1938	23
May 25, 1938	June 15, 1938	21
June 9, 1938	June 30, 1938	21
June 10, 1939	June 29, 1939	19
July 22, 1939	Aug. 11, 1939	20
July 25, 1939	Aug. 16, 1939	22
Aug. 12, 1938	Aug. 31, 1938	19
Sept. 1, 1938	Sept. 22, 1938	21
Sept. 4, 1939	Oct. 4, 1939	30
Oct. 15, 1938	Nov. 9, 1938	25
Oct. 29, 1938	Dec. 1, 1938	33
Nov. 19, 1938	Dec. 19, 1938	30

Figures 3, 4 and 5 are photomicrographs of embryos of *Z. lunata* at 13, 18 and 21 days of development respectively, from a culture in which the first miracidium hatched in 21 days. In the 13-day stage the embryo is 135μ to 140μ long and is slipper-shaped, occupying the entire length of the egg. The yolk masses have begun to increase in size and decrease in number by a process which suggests a coalescence of the contents of adjacent masses. Very little movement of the embryo could be observed at this stage. Cilia are present but were not seen to move. The anlage of the "gut" is well established and flame cells are only occasionally seen. They have large nuclei at the base of a small tuft of cilia which measures approximately 6μ $\times$ 3μ. The

nuclei are visible only after the embryonated egg is somewhat compressed and only after the embryo reaches a moribund state. No vitelline membrane could be discerned and from this stage on, the embryo occupies the entire length of the egg. No space is left at either end and the granular mass called a "mucoid plug" by Barlow (1925) and described in detail by Bennett (1936) for *Cotylophoron cotylophorum*, is entirely absent at all stages in eggs of *Zygocotyle lunata*. In *Z. lunata* numerous refractive, spherical masses are usually to be seen lying outside the embryo (Figs. 3, 4). They are left behind when the miracidium hatches and may be excretory in nature.

An 18-day old embryo (Fig. 4) shows practically all the body organs well developed and exhibits much movement, shifting backward and forward and constricting the body transversely at the junctions of the rows of epidermal cells. The yolk masses are now few in number and very large. Increase in length and width has necessitated a folding forward of the posterior fourth of the body, which becomes J-shaped. The nerve mass is well developed as a concentrated, somewhat spindle-shaped band of small cells near the posterior limit of the anterior third of the embryo. The cilia are active, beating spasmodically every few minutes. The flame cells are large (12 μ by 5.5 μ) and actively beating, but no collecting tubules were observed at this stage. Germ cells are well-defined in the posterior third of the body.

The embryo of 21 days development shown in Fig. 5 would not be ready to hatch before 24 to 48 hours although some others in the same culture dish were hatching. Several yolk masses are still present and appear to be partitioning off the part of the egg not occupied by the embryo. These apparent divisions always disappear completely before hatching occurs. At this stage there is much constricting of the body as a whole and of the "gut" which is half as long as the body. Granules suspended in a fluid in the "gut" shift back and forth due to apparent waves of contraction in the wall of the "gut" (Fig. 5). The cilia beat slowly from time to time. Anteriorly, the apical papilla presses closely against the operculum. The embryo maintains a J-shaped form and the position shown in Fig. 5 until the time of hatching.

Hatching. With the disappearance of all the yolk masses the content of the egg is a continuous fluid in which the miracidium lies free. It shifts back and forth rapidly and the cilia begin to beat violently. One gets the impression at first that the apical papilla batters against the operculum to open it by mechanical pressure. More gentle movements of the same nature take place during the last 2 days before hatching. In the majority of cases the miracidium emerges without having turned around in the egg and this appears to be the normal procedure. Shortly before hatching, many large refractive granules from 4 μ to 10 μ in diameter, and clusters of smaller spherules, remain more or less stationary within the egg shell, but when the miracidium is ready to emerge, the violent ciliary action causes the granules to be swirled rapidly about with the fluid. Normally the operculum opens within one hour after this violent activity starts and emergence of the larva requires from a few seconds to fifteen minutes. Those which require the longer time to get out of the egg shell are apparently abnormal in some way or are injured in emerging, since they usually do not swim far and soon die. The diameter of the operculum is only about half the width of the miracidium which must constrict appreciably as it passes through. During the entire process of emergence the cilia are beating very rapidly.

Some miracidia turn around again and again in the shell at hatching time. In such eggs, opening of the operculum appears to be delayed. Some of these larvae emerge normally when the operculum does finally open, but in other eggs the operculum opens only after the miracidium has exhausted itself by its activity and may emerge only half way and die in that position. Some swim around incessantly within the egg shell for as long as 7 hours and die there.

It seems significant that in many cases where the larva fails to emerge readily, it turns about actively in the egg and applies the apical papilla against the two ends of the shell with equal frequency, apparently trying to find an opening at first one end and then the other. In two such eggs observed in hanging drops, the operculum opened while the apical papilla was directed toward the opposite end of the egg. Within a few seconds the larva turned and emerged rather slowly through the opening but swam away vigorously when free from the shell. This observation seems to indicate that glandular secretions are produced at hatching time which effect the opening of the operculum, and that mechanical pressure by the apical papilla is probably not the direct cause for its removal. Normal larvae develop in a position adapted to straight-forward emergence. No embryos were observed developing in a reversed position.

A remarkable periodicity exists in the time of day that miracidia of *Z. lunata* hatch. In observations on hundreds of mature miracidia in many different culture dishes at all hours of the day and night, very few were observed to hatch or to have hatched before 5:00 P.M. and no miracidia were ever found swimming in the cultures in the morning after 9:00 A.M. The majority hatched between 10:00 P.M. and 2:00 A.M. In many cases, mature miracidia were observed in eggs until about midnight or later in order to study the hatching procedure. Often none would hatch, but almost invariably some of them would be found either moving very feebly or dead in the dish at 9:00 in the morning, having hatched during the early morning hours. Those which had not hatched during the night usually remained in the egg shell until the following night, when more would emerge. Darkness does not seem to be the controlling factor, for just as many larvae hatch in a culture dish placed under the strong beam of a microscope

lamp as in dishes covered with black paper. Attempts to induce consistent hatching of miracidia for study during the day, by the stimuli of light and darkness, were entirely unsuccessful. Agitation with dissecting needles of eggs containing mature miracidia sometimes causes the operculum to open, probably by mechanical injury to the egg shell, but most if not all individuals induced to emerge in this fashion are abnormal and die without getting very far away from the opened shell.

The miracidium swims very rapidly, usually in straight lines for considerable distances before darting off suddenly in a new direction. In syracuse watch glasses they may be seen either swimming back and forth across the dish or following around the edge in either direction. When no snail is in the dish the movements of the larva seem to be entirely at random. No phototropism was observed and they do not show any tendency to concentrate in any one place or in any way to influence each other. They swim incessantly for varying periods up to 7 hours, after which they slow down, sometimes swimming for a time in narrow circles, and die on the bottom of the dish in a somewhat bloated condition and contracted in length. As described in a later section of the present paper, they usually enter the snail host within two hours.

Morphology of the Mature Miracidium.

Studies on the morphology of mature miracidia of *Zygocotyle lunata* were conducted, using unstained living and moribund individuals in hanging drops, silver impregnated specimens prepared according to the method described by Lynch (1933), and specimens stained intravitally with neutral red. When swimming, the miracidium is elongated with sides almost parallel. Anteriorly it is cone-shaped and terminates in a protrusible cap called by some authors the apical papilla. The posterior end is bluntly rounded. Moribund specimens and those which may be momentarily quiescent contract appreciably in length and become broader. The anterior, coniform region and apical papilla may be retracted and the specimen becomes much expanded in the anterior half. Some variation occurs in size of miracidia of *Z. lunata* as in other species, but most of it is due to the varying amounts of contraction and swelling which occur on natural death or fixation. Ten specimens killed by hot 0.5 per cent. silver nitrate for the silver impregnation method showed proportions most closely approaching those of the living miracidium. In these 10 specimens length varied from 184 μ to 211 μ and width varied from 53 μ to 59 μ, the average length and width being 194 μ and 55 μ respectively (Figs. 7, 8). Ten moribund specimens in hanging drops varied from 170 μ to 231 μ in length and from 68 μ to 75 μ in width, the average measurements of this group being 187 μ by 69 μ.

The entire surface except the apical papilla and the very narrow spaces between rows of epithelial cells is ciliated. The cilia (Fig. 6) are about 10 μ long except immediately behind the apical papilla where they are about 3 or 4 μ long. These short cilia beat as do all the others. The cuticular non-ciliated cap or apical papilla is about 10 μ across and a slight constriction of the body wall occurs just behind it. The apical papilla, called by various authors a "rostrum," "oral cone," "head papilla," or a "terebratorium," is said by some to be perforated by small glandular pores. Lynch (1933) described such pores in the apical papilla of *Heronimus chelydrae*. Coe (1896) found an opening which he refers to as the mouth in the "Kopfpapille" of the miracidium of *Fasciola hepatica*. No pores were identified on this structure in *Z. lunata*, and such pores have not been reported on the papillae of other amphistome miracidia.

The external layer of the miracidium of *Z. lunata* is an epithelium consisting of 20 flattened, ciliated, epidermal cells. They are arranged in 4 tiers or rows, with 6 cells in the first (anterior) row, 8 in the second, 4 in the third and 2 in the last or posterior tier (Figs. 7, 8). These cells are well demonstrated by the silver impregnation technique used by Lynch (1933). Figure 7 is a photomicrograph of a silver impregnated miracidium taken with a 16 mm. objective to obtain sufficient depth of focus to show the entire thickness of the specimen. Figure 8 is a photomicrograph of the same miracidium as in Fig. 7 but using an 8 mm. objective focussed on the upper surface, with consequent loss of depth of focus.

The epidermal cells of the first tier covering the anterior fifth of the larval body are triangular in shape due to the tapering at the front end of the miracidium. They converge, almost meet, and seem to unite to form a ring in the base of the apical papilla. The cells of the second and third tiers are rectangular in surface view. Each of the 2 cells in the posterior tier covers one-half of the surface in the posterior fifth of the miracidium, the contiguous borders of the 2 cells lying somewhat to the left and right of the median plane. The spaces between adjoining epidermal cells and between tiers of cells in *Z. lunata* are from 0.5 μ to 1.5 μ in width as seen in silver preparations. The spaces are homogeneous in appearance, without perforations and are bordered by wavy, irregular lines. No overlapping of plates was observed. Papillae and excretory pores open laterally through spaces between epidermal cells, as will be described later.

The disposition of the epidermal plates with regard to the dorso-ventral axis of the miracidium is shown in Fig. 9, which is a dorsal view. Adjoining borders of the dorsal (and ventral) epidermal cells of the second and third rows lie in the median plane, while those of the posterior row lie in a plane about 45 degrees removed from the median plane. The borders of the 6 anterior cells do not fall in line with any of those of the second row of 8 cells, and the medial edges of the 2 dorsal epidermal cells in the anterior row lie just a little to one side of the median plane. The miracidium shown in the photomicrographs (Figs. 7, 8) lies in a position of about 45 degrees

of rotation to the right from a dorsal aspect, permitting favorable illustration of the lateral location of excretory pores and lateral papillae.

Epidermal cells have been studied in miracidia of a few other species. A review of this work was presented in tabular form by Bennett (1936). The cell formula, 6;8;4;2 was reported by Bennett (1936) for *Cotylophoron cotylophorum*, and by Krull & Price (1932) for *Diplodiscus temperatus*. The present work shows that the miracidium of *Zygocotyle lunata* also possesses the formula 6;8;4;2, indicating that this formula is probably characteristic for the family Paramphistomidae. In studies on these cells in miracidia from other families, Thomas (1883), Ameel (1934) and Lynch (1933) reported some variation between different individuals within a species. In the majority of species, however, in which the formula has been described, no variation is reported. In the present work, 25 silver impregnation preparations of miracidia of *Z. lunata* showed no variation in the formula for the epidermal plates. As pointed out by Price (1931) and Bennett (1936) these structures are probably of importance in establishing natural relationships among the trematodes.

Each epidermal cell contains a nucleus which may sometimes be seen in moribund or unstained dead miracidia, and may be best observed with the aid of intra-vitam stains. They do not show in silver impregnated preparations. In the present work they were studied from surface views of whole mounts, either unstained or stained with neutral red. The nuclei of the first tier of epidermal cells are much elongated, measure from 14 μ to 17 μ in length and about 2 μ in width, and lie very near the posterior border of the cells. Each of the 8 epidermal cells in the second tier contains an elongated but somewhat irregular-shaped nucleus near its posterior border (Fig. 9). These nuclei are 11 μ to 13 μ in length and 3 μ in width. Those of the third tier of epidermal cells show the same position, shape and length but are only about 2 μ in width. Each of the 2 posterior cells contains a centrally located nucleus about 14 μ long and 5 μ wide.

As pointed out by Bennett (1936), the nuclei of the epidermal cells have been described for only a few miracidia. With slight variations in shape, size and position, these structures are apparently similar in the miracidia of all the Paramphistomidae in which they have been investigated. In this group Sinitsin (1931) described them in the miracidia of *Paramphistomum cervi*, Krull & Price (1932) in *Diplodiscus temperatus* and Bennett (1936) in the miracidia of *Cotylophoron cotylophorum*.

Beneath the surface layer of ciliated epidermal cells is a layer of transparent subepithelial cells. As indicated by their nuclei, these cells form a continuous layer around the internal structures of the miracidium. The nuclei are distributed over all portions of the subepithelial layer, but are more numerous in the anterior half of the body (Fig. 9). In an optical section of a whole mount viewed from the dorsal side, from 13 to 15 subepithelial cell nuclei may be seen along each outer edge of the miracidium. In surface view a group of from 12 to 15 may be seen overlying the "primitive gut" and smaller groups may be identified in the middle and posterior regions of the body. A few of these nuclei appear spherical but most are slightly elongated and vary from 6 μ to 7.5 μ in length and from 4 μ to 5 μ in width. Krull & Price (1932) showed that in the miracidium of *Diplodiscus temperatus* these nuclei are arranged in 3 definite rows, and Bennett (1936) found them to be distributed in 4 principal groups in the miracidium of *Cotylophoron cotylophorum*. Bennett pointed out, however, that not all the nuclei are to be found in these groups. In *Zygocotyle lunata*, the subepithelial nuclei are distributed irregularly through the subepithelial layer, and while they are more numerous in some areas than in others no definite arrangement into groups could be observed. Actually, examination of different specimens of *Z. lunata* showed considerable variation in number and distribution of the nuclei. Bennett (1936) observed mitosis in some of the subepithelial nuclei in *C. cotylophorum* and he points out the futility of attempting to determine their number.

The "primitive gut" is a saccate or flask-shaped structure occupying a considerable portion of the anterior region in the miracidium of *Z. lunata*. Its shape is exceedingly variable both in developmental stages within the egg (Figs. 4, 5) and after hatching of the larva. It may be elongated and narrow or it may be constricted transversely, or it may shorten and become very broad. When elongated it extends past the middle of the body and when shortened it occupies only the first third of the body. No lumen in the ordinary sense of the term is present and it is completely filled with a fluid containing a coarsely granular material which surges back and forth actively due to contraction of its walls and of the body of the miracidium. Anteriorly it tapers and seems to terminate blindly just behind the apical papilla. No opening to the outside could be found and nothing was observed to be either taken in or extruded from it. Its walls show no cell boundaries in the mature miracidium. Four large, somewhat ovoid nuclei, measuring about 8 μ long by 5 μ wide, are situated near the posterior end, where they may be seen at all times since they remain attached to the wall and do not surge about with the granular contents of the sac (Figs. 5, 9).

Most helminthologists have considered the "gut" of the miracidium as a primitive or vestigial intestine but more recently the work of Reisinger (1923), Manter (1926), Price (1931), Lynch (1933), and Bennett (1936) seems to suggest that this structure is a gland rather than a gut. Bennett (1936) says, "The development of the primitive gut at some distance from the anterior end of the body, the size of the cells and their nuclei, the early development of the granular contents, the absence of a definite cell wall around each nucleus after the four-cell stage is reached, the concentration of cytoplasm around

the nuclei at the posterior end of the gut, the absence of a mouth and a lumen, and the complete disappearance of the contents immediately after penetration of the miracidium into the snail host while the nuclei may still be identified—all give evidence in favor of interpreting this structure as being a gland rather than a gut."

The writer is inclined to agree with the opinions of the above-mentioned workers that the so-called "primitive gut" is probably glandular in its function, but more intensive studies on the embryonic origin and the fate of this structure will have to be conducted before final decision can be made as to its nature. If it functions in the penetration of the larva into the snail host, an opening must develop at that time, and the granular contents may consist of secretory granules formed in a structure which might be homologous with a gut in other forms.

The excretory system of the miracidium of *Z. lunata* resembles that described for other members of the Paramphistomidae. Two large flame cells, one on each side, measuring from 13 μ to 15 μ in length of flame and from 6 μ to 7 μ in width, are located just anterior to the middle of the body (Figs. 6, 9). Each possesses a large spherical nucleus at the base of the flame. A collecting tubule passes posteriorly in loose coils from each flame cell to a level behind the excretory pores, then loops forward to encircle the flame cell and again passes back to open at the excretory pore, which is located laterally just in front of the junction of the third and fourth tiers of epidermal cells (Fig. 8). A large spherical vesicle lies just anterior to each flame cell in the miracidium during late development in the egg as well as after hatching (Figs. 5, 6). However, no morphological association could be established between them and the excretory system. No accessory excretory cells as described for the miracidia of *Heronimus chelydrae* by Lynch (1933) and no duct nucleus as reported for that of *Diplodiscus temperatus* by Krull & Price (1932) could be found in the miracidium of *Z. lunata*.

Krull & Price (1932) and Bennett (1936) reported 2 pairs of penetration gland cells in the miracidia of *D. temperatus* and *C. cotylophorum* respectively. They described these glands as 4 unicellular units extending from the base of the apical papilla backward for about one-fifth of the body length, with nuclei at their posterior extremities. From the descriptions and figures, the 4 ducts which open anteriorly are each a part of one of the 4 gland cells. No indication is given concerning their role in penetration. In the present material of *Z. lunata*, 2 nuclei with clear areas around them were found on each side at a level near the junction of the first and second rows of epidermal cells. These nuclei, slightly ovoid in shape, were about 4 μ in length. In spite of repeated observations on different miracidia at different stages of development, it was not possible to find any ducts leading anteriad from these clear spaces around the nuclei.

The greater portion of the posterior half of the miracidium is occupied by germinal tissue, which consists of about 40 germ cells as evidenced by their large spherical nuclei which measure from 5 μ to 8 μ in diameter. In all miracidia observed after hatching, one or more germ balls were present in addition to the germ cells. Most of the larvae contain one large germ ball measuring 25 μ across and consisting of about 16 cells with a definite membranous covering. This germ ball lies in the anterior half of the body behind the "gut" and between the flame cells. In some specimens an additional smaller germ ball could be identified. The germ balls and the larger germ cells lying in the central cavity of the miracidium appear to be completely free and not attached to any other structure.

The nervous system consists of nerve cells and fibers in association with a large ovoid mass lying dorsal to the "gut" and sending out processes laterally and posteriorly. Other processes probably extend forward but these were not observed. The dorsal nerve mass contains cells and fibers and measures about 25 μ by 30 μ. Large lateral processes could be observed in living miracidia while confined in the egg as well as after hatching. They could be traced to the body wall and were then lost. Very small cells, visible only with the aid of intra-vitam stains, lie scattered about outside of the central nerve mass.

Two papillae protrude laterally through openings between the ciliated epidermal cells at the level of the posterior border of the first tier of epidermal plates (Figs. 7, 8). They have been observed in numerous other species and have been variously called lateral papillae, anterior papillae, lateral processes and anterior ducts by different authors. They are probably sensory in function since in some forms they are described as being associated with the central nerve mass. They are about 6 μ in diameter and their position between the epidermal plates is clearly shown in silver impregnations by large round spaces. Smaller but similar spaces appear at other points of union between the first and second tiers of cells, but no structural units associated with them could be identified in *Z. lunata*. Lynch (1933) observed a number of small motionless bristles in this position in the miracidia of *Heronimus chelydrae*.

Experimental Infestation of the Intermediate Host.

The intermediate host in the life cycle of *Zygocotyle lunata* is the snail *Helisoma antrosum*. Naturally infected snails of that species provided the cercarial stage from which the adults were obtained and the complete life cycle demonstrated. Laboratory-raised snails were experimentally infected with miracidia developed in eggs from feces of experimentally infected ducks and rats. *Helisoma antrosum* reproduces readily in the laboratory at all seasons of the year and was available in stock tanks at all times. All snails used in the infection experiments were laboratory-bred. Miracidia were strongly attracted to the snails, to pieces of snail or to snail

feces. To collect the rapidly-swimming miracidia from a dish, it was only necessary to place a snail in the dish and by the time one could place the dish on the stage of the microscope and focus on the snail, the miracidia would have gathered around it. They swim under and over and around the shell, occasionally attaching momentarily to the foot, edge of the mantle or to the shell, and then break away again and attach at some other point or even swim away to a different snail. If a snail is removed from the dish before penetration has occurred, the miracidia are attracted by the mucus left behind by the snail. No response to light could be observed. If no snail is present in the dish, the miracidia swim for periods up to 7 hours, settle to the bottom of the dish, and they may swim there in a narrow circle for a short time until death ensues.

Miracidia were observed with snails for varying lengths of time. In some cases miracidia disappeared under the shell within 15 minutes. Long and careful search failed to find them again and either they had penetrated or had been caught in the mucus secreted by the snail. This procedure was observed repeatedly, the time required for this apparent penetration varying from 15 minutes to 2 hours. In two cases, miracidia were seen to enter the space within the shell and attach to the base of the foot in a position perpendicular to it. Little progress, if any, toward penetration had occurred after one hour when observation was discontinued.

In all the early attempts to infect *Helisoma antrosum*, medium sized snails from 8 to 10 mm. in diameter were exposed individually in separate finger bowls to 1 to 15 miracidia. Nineteen snails were thus exposed between January and June, 1938, but none of the snails became infected. After observing the apparent penetration of the larvae into the snails, successful infections were fully expected and no explanation could be offered for the negative results. On June 29, the writer, preparing to leave for Woods Hole, dumped about 40 eggs which were due to hatch 2 days later, into a battery jar containing some very young laboratory-raised snails. Another batch of 70 eggs of *Z. lunata* due to hatch July 8 was placed in a 2-gallon aquarium jar containing laboratory-raised snails of various sizes. Returning to the laboratory on August 17, the writer found encysted metacercariae on the glass in both jars. Isolation of the 42 snails showed 9 infestations, all but one of which were in snails 7 mm. or less in size. The single larger infected snail was 13 mm. in diameter.

On August 27, 20 eggs of *Z. lunata* with miracidia ready to hatch were put into a finger bowl with 3 very young snails, each being about 2 mm. in diameter, and 35 days later 2 of the 3 snails began giving off cercariae. Young snails from 2 mm. to 6 mm. in size were used in all later experimental infections. Mass infections using large numbers of miracidia in small aquarium jars containing from 20 to 75 snails were much more successful than when a single individual was exposed to a few miracidia in a separate dish. In such mass infections, from 10 per cent. to 55 per cent. of the snails became infected. Not less than 80 successful experimental infestations of *Helisoma antrosum* were obtained in which mature cercariae were produced. Many other experimentally infected snails were killed for dissection or sectioning during the early stages of the infestation. The miracidium metamorphoses in the snail into a sporocyst which in turn produces rediae. The redial generation gives rise to cercariae which emerge from the snail from 32 to 49 days after penetration of the miracidium.

The snail host may carry an infestation with *Z. lunata* for long periods. The 4 naturally infected snails from which the life history studies began were collected in September and October, 1937, at which time they were giving off cercariae in numbers up to 100 per day. Of these, one died on December 29, 1937, 2 others died in April, 1938, and the 4th, which was recorded as a light infestation the previous September, lived until June 25, 1938. During this period they were kept isolated in finger bowls and fed lettuce leaves, and the water was changed about once a week. Many thousands of cercariae were produced. Some of the experimental infections persisted equally long. From a group of 12 snails which began giving off cercariae on December 18, 1938, seven were still living in the laboratory and shedding cercariae after 9 months. From a size of not more than 5 mm. when infected they had grown to an average size of 15 mm. in diameter of shell. No snails were exposed to miracidia a second time after they were once infested with *Z. lunata*. None was observed to have lost the infestation and those which were crushed or died naturally after giving off cercariae for 9 months still contained large numbers of immature and mature rediae and cercariae. The larvae infest most heavily the liver and gonads of the snail host but rediae and immature cercariae are found in considerable numbers in the lymph spaces and practically everywhere in the snail except in the lumen of the intestine. None of the snails produced any eggs after becoming infected. As seen in sectioned snails, the gonads are reduced to a few shreds of tissue or cannot be identified at all.

Sporocyst.

The sporocyst of *Z. lunata* was observed only in the mature condition. Attempts to find early sporocysts by dissection of snails within a few days after exposure to miracidia were unsuccessful. Mature sporocysts were found in snails sectioned from 22 to 28 days after penetration of the miracidium. At this stage many rediae and immature cercariae are already free in the tissues of the snail. Usually no sporocysts can be found in an infected snail which has begun to shed cercariae, but one snail dissected 47 days after infestation, yielded a sporocyst measuring 297 μ in length and 195 μ in width which contained a single well-developed redia and nothing else. On manipulation of the coverslip the wall was ruptured and the young redia emerged. All other

snails dissected later than 28 days after infestation failed to yield sporocysts. No sporocysts of *Z. lunata* were found in the naturally infected snails studied.

The mature sporocyst varies in shape from ovoid to elongate and is broader anteriorly than at the posterior end. It is a simple saccate structure with a body wall consisting of a cuticle, a membranous sheet and muscle fibers. Within the central cavity are germ balls and young rediae in various stages of development. The excretory system is that which is carried over from the miracidium and consists of a pair of flame cells with collecting ducts which open laterally a short distance behind the middle region of the body. A terminal bladder was observed on each side.

A mature sporocyst found in sections of a snail killed 28 days after penetration of the miracidium contained 2 germ balls at the posterior end and 5 young rediae which occupied the central cavity. A reconstruction of this specimen is shown in Fig. 10. Each of the contained rediae shows a well-developed pharynx and an intestine which occupies most of the space within the young larva and extends nearly to its posterior border. The pharynx varied in diameter from 23 μ in the 2 individuals in the posterior region to 27 μ in the young redia near the anterior end. Figure 14 is a photomicrograph of one of the sections of this sporocyst and shows parts of 4 of the 5 rediae. The pharynges shown are those of the two posteriorly placed rediae.

Redia.

The redial generation of *Zygocotyle lunata* was described by Willey (1936) in a paper on *Cercaria poconensis*. Since *Cercaria poconensis* Willey, 1930, is the larva of *Z. lunata*, the mature redia need not be again described here. However, since only naturally infected snails were available for the previous study, no information was obtained on the early stages of infestation. With an abundance of material from experimentally infected snails it has been possible in the present investigation to make more complete observations which reveal the presence of daughter rediae.

In the earlier description (Willey, 1936) of the stages found in the snail, it was stated that, "All the snails examined showed very many rediae of all sizes, but no sporocysts or mother rediae were found." Many very small rediae as well as larger ones are always to be found in crushed infected snails, even in those killed 9 months or longer after experimental or natural infestation. This fact indicated strongly the existence of two generations of rediae, since the sporocyst disappears early in the course of the infestation. Accordingly, in the present study, experimentally infected snails were dissected or sectioned at different periods following penetration of the miracidium of *Z. lunata*. Most of the observations were made on living material from crushed snails, but sections of infected snails also show all the more important stages.

In some of the snails crushed during early stages of the experimental infestations, mother rediae were found which contained only a single daughter redia and a few undeveloped germ balls near the posterior end. A specimen of this type shown in Fig. 11 was 330 μ long and its pharynx measured 33.4 μ in diameter. The daughter redia was 198 μ in length and its pharynx also measured 33.4 μ in diameter. The young redia showed considerable movement within the parent and its intestine extended back more than three-fourths of the length of the body. In this early stage of infestation no cercariae were as yet to be found free in the tissues of the snail, but rediae were also present which contained only developing cercariae (Fig. 15). In a group of snails crushed 20 days after experimental infestation, numerous rediae were present which contained a single daughter redia and 3 to 6 germ balls which were not as yet differentiated. In no case could more than a single daughter redia be identified within a parent redia.

Infected snails, killed 25 days or later after penetration of the miracidium, contained mother rediae with one daughter redia and numerous immature cercariae. Few such mother rediae were present in any one snail, but all snails examined between 25 and 47 days following infestation contained this stage in addition to many rediae in which only cercariae could be observed. The identification of the different larval stages within the same mother redia was unmistakable. The single daughter redia seen in Fig. 13 showed much movement, squirming and turning around actively. The several cercariae present were larger and showed no movement. The well-defined pharynx in the daughter redia measured about 30 μ in diameter, while the oral sucker of a developing cercaria is much larger, when it first becomes definable, than the pharynx of a large mature redia. Further, in some cases the eye-spots of the cercariae had begun to develop. The cones of cilia in the flame cells of daughter rediae are from 11 to 13 μ in length, whereas those of cercariae of this species are only from 5 to 7 μ long.

Mother rediae containing both a daughter redia and cercariae were found in laboratory-infected snails studied during the period from August to March, but it seems probable that rediae are produced singly by mother rediae throughout the course of the infection. This is indicated by the fact that infected snails at all stages of infestation always contain great numbers of mature and immature rediae of various sizes free in the tissues. They must be produced more or less continuously in the snail host and emerge from the parental generation at a relatively early stage. All daughter rediae observed were located in the anterior region of the mother rediae and they are always produced singly. Rediae containing only a daughter redia and no cercariae (Fig. 11) are small and are found only in the early stages of the infestation. Rediae containing both a single daughter redia and cercariae were all older and larger individuals

measuring from .7 mm. to .9 mm. in length. The redia shown in Fig. 13 was .792 mm. long and contained a daughter redia measuring .335 mm. by .066 mm., while that in Fig. 12 was .860 mm. in length and contained a much younger daughter redia which measured .172 mm. in length and .063 mm. in width. These facts suggest the probability that each redia produces one daughter redia and proceeds from that time on to produce cercariae only. This would explain the presence of rediae of all sizes during all stages of the long-term infestation in this species.

The rediae move sluggishly when freed from the snail, and in a watch glass usually remain in one place with only slight changes in body shape due to muscular contraction. No locomotor appendages are present at any stage. Twelve flame cells are present in the mature redia of *Z. lunata* while only 6 appear in younger individuals. Most amphistome rediae described show a total of only 6 flame cells in the excretory system of the mature redia. Looss (1892) mentions 4 flame cells on one side in the redia of *Diplodiscus subclavatus* and Krull & Price (1932) figure a total of 7 in that of *Diplodiscus temperatus*. Looss (1896) described 5 pairs of flame cells in the mature redia of *Paramphistomum cervi*.

A daughter generation of rediae has been described for several species of amphistomes. Looss (1896) reported more than a single generation of rediae in *Gastrodiscus aegyptiacus* and in *Paramphistomum cervi*. In life history studies on *P. cervi*, neither Takahashi (1928) nor Szidat (1936) were able to find any evidence for a second generation of rediae. Beaver (1929) described 2 generations of rediae in *Allassostoma parvum*. Le Roux (1930) indicated that mother rediae occurred in the life cycle of *Cotylophoron cotylophorum* and Bennett (1936) found one specimen of a mother redia in that species. Krull & Price (1932) reported only one redial generation for *Diplodiscus temperatus*, but Herber (1938) demonstrated both mother and daughter rediae in that species. The development of rediae and cercariae within the same mother redia is known for only one other species of amphistome, and the present work constitutes the second report of this condition in amphistomes. Looss (1896) described a redial generation of this type in *Gastrodiscus aegyptiacus*. He figures a redia containing 3 daughter rediae and 3 cercariae and states that rediae containing only cercariae are rarely encountered in that species.

Some authors have postulated that in certain forms a definite number of rediae and cercariae are produced. In the absence of experiments with known numbers of miracidia infecting the snails, no definite conclusions can be reached in this regard in any species of trematodes. In *Zygocotyle*, the finding of only small numbers of mother rediae containing single daughter rediae and cercariae together indicates that the production of rediae is a continuous process and is not limited to a definite number. Variations may occur in the redial generations in the snail under natural conditions accompanying seasonal changes. Experimentally infected snails kept under laboratory conditions do not always afford sufficient information on which to base conclusions concerning the prevalence and numbers of the various larval stages. The course of events probably varies with changing conditions. Much work still remains to be done on these problems of development.

Cercaria.

The cercaria of *Zygocotyle lunata* was named *Cercaria poconensis* in an abstract by Willey (1930), and in 1936 he described the redial and cercarial generations together with a report of infestation experiments with the metacercarial stage. The cercaria need not be redescribed here and only additional information not available from the earlier studies will be presented. The material from which *C. poconensis* was described and that from which the life history of *Z. lunata* is demonstrated in the present study are unmistakably identical. The size and the shape and distribution of the body organs in the two groups of specimens agree perfectly. The size measurements vary somewhat with different degrees of flattening. For example, the oral sucker in fixed and unflattened cercariae shows an average measurement of .064 mm. in diameter, while in living specimens, flattened under medium pressure, it may be as large as .106 mm. across. The oral sucker and oral evaginations together measure .165 mm. in length in fixed, unflattened individuals.

Näsmark (1937), in a revision of the Paramphistomidae, has attempted to show that the sucker at the anterior end in amphistomes is a pharynx rather than an oral sucker. On the basis of histological studies, he believes that this structure is homologous with the pharynx of the monogenetic trematodes and the rhabdocoel turbellarians, and states that contrary to the opinion of Looss (1902) and others it should be designated a pharynx. However, due to the incomplete nature of Näsmark's evidence, the conclusions are not accepted by the present author and the sucker is here referred to as an oral sucker. Fischoeder (1903) referred to the anterior sucker of *Z. lunata* as a pharynx, causing some misinterpretation in the literature, but all the more recent authors have preferred to call it an oral sucker. Until final conclusive evidence is forthcoming, the terminology currently used seems more desirable.

The posterior overhanging lip of the acetabulum with its 2 lateral conelike projections (Figs. 21, 22), which are so characteristic of the genus *Zygocotyle* in the adult stage, were not observed in the earlier studies on the cercarial stage. But after it became known that this cercaria was the larval stage of *Zygocotyle*, the lip and conical projections were observed from the ventral surface of cercariae when under only slight pressure. This modification of the acetabulum is present on the cercaria in an immature condition. Figure 16 is a photomicrograph of a living cercaria under medium pressure and shows the

relationships of the various organ systems. The characteristic branching of the main excretory ducts is clearly outlined by the presence of excretory concretions. Drawings from both living and fixed material of redial and cercarial generations appear in the earlier description (Willey, 1936).

The cercaria leaves the redia while still in a very immature condition. The intestine, oral sucker, acetabulum, eye-spots, tail rudiment and the excretory system, already laid down while the cercaria is in the redia, continue their development after emergence. Some variation occurs in the size and degree of differentiation of the cercariae at the time of emergence. Young cercariae are frequently observed free in the tissues of the snail which have not developed as far as other cercariae which are still within nearby rediae.

In experimental infestations, young cercariae were first found free in the tissues of snails crushed 16 days after exposure to miracidia. In other snails, crushed 20 days after exposure to miracidia, none had as yet emerged from the rediae. The rate of development is known to vary with temperature conditions. The time required for the complete development of the cercaria, from penetration of the miracidium until shedding of the first mature cercaria from the snail, varied in the laboratory from 32 days during the warmer months to 49 days during the winter.

After emergence, cercariae swim about vigorously in the water at the side of the container toward the light for 30 minutes to 2 hours. They respond very rapidly to changed lighting conditions and will follow a beam of light moved about from one side of the dish to the other. Encystment occurs on the side of the dish toward the light, on the shell of the snail, or occasionally on vegetation. If handled or otherwise irritated they encyst almost immediately, often attaching and encysting within a dropper when being transferred to a slide for examination. If placed on a slide in a small amount of water, a coverglass must be added immediately to prevent encystment. The shell of a snail producing cercariae is usually crowded with encysted metacercariae. Cercariae emerge in greatest numbers between 10:00 A. M. and 2:00 P. M. and usually only on bright days. On dull, rainy days very few or none escape, while on bright, sunny days as many as 100 may emerge from a single infected snail at the peak of the infestation. Cercariae may be produced from an infected snail for 9 months and longer, but the number of cercariae escaping each day decreases as the snail remains longer in the laboratory. Little difference could be observed between the numbers of cercariae produced in naturally and experimentally infected snails.

Metacercaria.

The cercaria encysts and passes into the metacercarial stage in which it awaits ingestion by the final host. The process of encystment is very rapid. The cercaria attaches itself by means of its suckers, the tail vibrates from side to side somewhat more slowly than in swimming, and the body appears to undergo rapid squirming movements. Cystogenous material then oozes out rapidly over the surface of the body from the elliptical cystogenous granules which occupy most of the dorsal half of the cercaria. The cyst wall forms rapidly and the tail is left attached to the outside of the cyst, where it lashes violently for an hour or more and then drops off, sinks to the bottom and may continue lashing about for several hours. The body of the cercaria twists and turns about during the process as though molding the inner wall of the cyst. Finally the cyst wall hardens, the metacercaria coils about in the cyst and after several hours becomes relatively motionless with suckers apposed, periodically undergoing slight twitchings and contractions in various regions of the body.

The cysts are large dome-shaped hemispheres with thick resistant walls which are brown to black in color when seen with the naked eye. The base of the cyst flares out slightly from the margin of the cyst proper (Fig. 18). When formed on glass they are flat on the bottom and the greatest diameter shows an average measurement for 10 metacercariae of .368 mm. The diameter at the base of the dome varies in 10 individual cysts from .277 mm. to .343 mm., the average being .289 mm. Figure 19 is a photomicrograph of a 10-day old metacercaria which has been dissected from its cyst. When removed thus in water or salt solution, they contract and undergo little or no movement. Much black pigment is present in the body wall and this tends to obscure the internal structures, which show no advance in development over the condition found in the cercaria. The black pigment of the eye-spots and body persists throughout metacercarial life and scattered granules of eye-spot pigment are still present after 3 weeks of development in the final host (Figs. 21–23).

Infestation Experiments with Metacercariae. Early attempts to infest the final host with the metacercaria of *Z. lunata* were conducted, using cold-blooded hosts. As outlined by the writer in an earlier paper (1936), encysted metacercariae were fed to tadpoles but all the experiments were negative. In 1937, following unsuccessful attempts to infest turtles with the larvae, some of the metacercariae were dissected from their cysts and placed in cold Ringer's fluid at 20° C. All were dead after 3 hours. Artificial digestion of some of the metacercariae from their cysts with pepsin and pancreatin solutions at 37° C. indicated that the final host was a warm-blooded animal. Under this treatment the cyst walls became soft and movement of the metacercaria was observed after 4 hours. After 10 hours and up to 20 hours of artificial digestion, young worms were still alive in the cysts while others showed much activity on being released from the cyst.

Consequently on October 8, 1937, 2 young laboratory-raised rats were each fed about 65

metacercariae obtained from the naturally infected snails as described earlier in the paper. Five days later one of the rats was killed and yielded 59 young worms in the cecum with none above or below this level in the intestine. Development had proceeded far enough to permit identification of the worms as *Zygocotyle*. The small cone-like projections on the posterior edge of the acetabulum are well developed at this stage (Fig. 20). The worms, still immature, show average measurements for 10 of them of .940 mm. in length and .460 mm. in width. The common natural hosts of *Zygocotyle* are various species of ducks. Thirteen young ducks were subsequently fed varying numbers of cysts and all became infected. Similarly, in the course of the investigation, more than 60 rats and one ram were experimentally infested. Attempts to infect pigeons and rabbits gave negative results.

In order to determine the time required for the young worms to mature in the final host, fecal examinations were conducted almost daily on some of the experimental hosts. Two ducks, (nos. 1 and 3) fed metacercariae on October 14, 1937, began giving off eggs of *Zygocotyle* in fecal material on November 24, or on the 41st day of infestation. Duck number 3 was killed 5 days later and contained 7 mature specimens of *Zygocotyle lunata*, 5 in one cecum and 2 in the other. Similarly 4 rats (nos. 3, 4, 5 and 6) were fed from 30 to 40 encysted metacercariae each on October 14, 1937. On November 24, when the 2 ducks described above showed eggs in the feces, the rats were still negative. One rat, number 5, killed on November 28, the 45th day, contained 23 *Zygocotyle*, several of which showed a few eggs in the uterus near the ovary, but none was fully mature as yet. Eggs of *Z. lunata* from this series of rats first appeared in the feces of rat number 6 on the 46th day after infestation. This rat was killed on December 4, the 51st day after the experimental feeding, and contained 8 fully mature *Zygocotyle lunata* in the cecum. The more rapid development in the duck may possibly be due to the higher body temperature maintained by that host. This difference was confirmed in other series of infected rats and ducks, the data for which will be described later.

Thus, beginning with cercariae from naturally infected snails, all stages in the development of the adults of *Zygocotyle lunata* were obtained in rats and ducks. As described in the earlier sections of the present paper, eggs from feces of the experimental hosts were embryonated to produce miracidia which were experimentally introduced into laboratory-raised snails. In the snail, *Helisoma antrosum*, the sporocyst and the redial and cercarial stages were obtained and the complete cycle was carried out several times in the laboratory.

Experiments on Infectivity of Metacercariae. After encystment the metacercaria remains quiescent and awaits ingestion by the final host. No development occurs within the cyst. Metacercariae dissected out or freed from their cysts by artificial digestion after one day of encystment are in no way distinguishable from those similarly freed after 20 or 30 days. This observation was confirmed by feeding experiments with rats. Cysts in a few drops of water were placed on small pieces of bread and given to the rats after withholding all food for 24 hours. In an experiment conducted on December 30, 1937, rat 22 was fed 100 metacercariae which were 14 days old and rat 25 was fed 100 metacercariae which were 85 days old. Both rats were killed 15 hours after ingesting the larvae and each rat yielded more than 30 excysted worms in the cecum. Some larvae were still within their cysts and none could be found anywhere in the digestive tract other than in the cecum. No significant difference could be detected between the two groups of young worms. All were equally active and the number of worms found was approximately the same in the two hosts. In an experiment designed to detect any changes which might occur immediately after encystment of the metacercaria, rat 12 was fed 50 newly encysted larvae, some with the cercarial tail still attached and the cyst wall still soft, and rat 11 was fed 70 metacercariae which were 47 days old. Both rats were killed 3 days later and yielded young worms in the ceca which were practically indistinguishable morphologically in the two hosts. Thirty-five worms were collected from rat 12 which received 50 newly formed cysts and 60 worms were obtained from rat 11 which received 70 cysts 47 days old. A few cysts are probably destroyed by the rat in the chewing of the bread. In a third experiment, one rat (no. 23) was fed 15 metacercariae which were 2 days old and 15 which were 32 days old. This rat was killed 6 days later and the 20 young worms collected from the cecum showed no significant differences in size or degree of differentiation. The rate of development of *Zygocotyle lunata* to sexual maturity is not correlated with the age of the metacercaria at the time of its ingestion. These experiments show conclusively that no development occurs in the cyst of *Z. lunata* and also that the encysted metacercariae are infective immediately after encystment.

Longevity. In one of the foregoing experiments, metacercariae, encysted for 85 days, were viable when fed to rat 25. In a series of longevity experiments, it was determined that the metacercaria will live much longer than that. Encysted metacercariae were kept at room temperature in the laboratory in covered finger bowls attached to the glass wall where first deposited, and the water was not changed except to add water occasionally to make up for evaporation. Metacercariae not older than 3 months were viable and when fed to rats produced infestations with *Z. lunata*. Encysted larvae older than 4 months gave variable results (Table 2). The oldest metacercariae which successfully produced an infection in rats had been in the laboratory for 138 days. In this experiment rats 48 and 49 were each fed 50 metacercariae from cercariae emerged from snail number 2 on January 30, 1938. Movements of the larvae within

Table 2.

Longevity of encysted metacercariae of *Zygocotyle lunata.*

Host and date of feeding metacercariae	Age of metacercariae in days	Number of metacercariae ingested	Elapsed time before killing host (Days)	Number of worms recovered	Remarks
Rat 34 June 2, 1938	86	10	11	3	
Rat 35 June 2, 1938	86	10	11	8	
Rat 30 Feb. 3, 1938	121	100	46	0	Movement observed in cyst before feeding to rat
Rat 29 Feb. 3, 1938	131	100	46	0	Movement observed in cyst before feeding to rat
Rat 46 June 9, 1938	130	50	9	23	Movement observed in cyst before feeding to rat
Rat 47 June 9, 1938	130	50	9	28	Movement observed in cyst before feeding to rat
Rat 48 June 17, 1938	138	50	169	1	Eggs in feces after 74th day
Rat 49 June 17, 1938	138	50	169	2	Eggs in feces after 74th day
Rat 63 Feb. 10, 1940	152	50	14	0	Cysts shrunken Movement in 10%
Rat 44 June 4, 1938	166	150	9	0	Cysts not examined
Rat 45 June 4, 1938	166	150	9	0	Cysts not examined
Rats 64, 65, 66, 67 Feb. 29, 1940	171	25 each	5–10	0	Cysts shrunken No movement observed

the cyst were observed before feeding. Seventy-four days later many eggs of *Z. lunata* were collected from feces of both rats and when these 2 rats were killed after 6 months of infestation, large, adult specimens of *Z. lunata* were recovered. In a similar experiment with rats 46 and 47 (Table 2), metacercariae 130 days old were fed and when the rats were killed 9 days later, 23 young worms were recovered from the cecum of rat 46 and 28 from rat 47. As indicated in Table 2, cysts 152, 166 and 171 days old respectively were fed to rats but no infestations resulted.

Some variation in longevity occurs. In the experiment involving rats 29 and 30, no infection was obtained from metacercariae aged 131 and 121 days respectively, although movement was detected within the cyst. Minor variations in other factors would readily account for such slight variations in longevity and infectivity. That these are not due to individual differences in the host reaction is indicated by the fact that in most of the experiments dealing with longevity of metacercariae, 2 host animals were fed similar numbers of larvae produced from the same snail host on the same dates, and in each case the results were always the same in the 2 hosts concerned.

No evidence is available as to what constitutes optimum conditions for long survival of encysted metacercariae. Bennett (1936) reports that under optimum conditions the metacercaria of *Cotylophoron cotylophorum* probably lives for several months. In his experiments, he presumably kept them at room temperature and after 3 months (June 5 to September 5) 33 per cent. were still alive. At this point observations were discontinued. Krull (1934) kept metacercariae of the same species alive under the same conditions for as long as 5 months from July 2 until December 2. Both of these workers used the criterion of movement of the metacercaria to determine its life span. As indicated by the data on rats 29, 30 and 63 (Table 2), movement in the cyst shows the metacercaria of *Z. lunata* to be alive as long as 5 months, but such larvae, when fed to the final host, were in these three attempts unable to survive conditions within the final host. It seems probable that motility is not a valid criterion of infectivity.

Experimental Studies on Viability of Metacercariae. Experimental feedings conducted with rats show that at least 85 per cent. of the metacercariae of *Z. lunata* excyst and survive for a time in the final host. The percentage of viable metacercariae is probably higher since some of the cysts may be destroyed in the chewing process by the rat host. The number of metacercariae which have excysted and are recoverable as young worms is seen to be very high if the host is killed within the first few days after ingestion of the cysts, because, as will be shown later, the host may begin to lose some of the worms as early as the second or third day. Table 3 shows the number and percentage of worms obtained in 10 experiments in which the hosts were killed within the first 11 days. The high percentage of viability of metacercariae is shown in feeding experiments with ducks as well as rats. For example, duck 3a, fed 150 encysted larvae and killed 11 days later, yielded 144 young worms in the ceca.

TABLE 3.

Viability of encysted metacercariae of *Zygocotyle lunata*.

Host	Metacercariae ingested by host: Number	Metacercariae ingested by host: Age (Days)	Days elapsed before killing host	Number of worms recovered	Percentage of worms recovered
Rat 7	90	12–18	2	85	94.4
Rat 11	70	47	3	60	85.7
Rat 12	50	0–2 hrs.	3	35	70
Rat 1	65	8	5	59	90.7
Rat 23	30	2–32	6	20	66.6
Rat 6a	60	1–3	7	55	91.6
Rat 6b	80	30	7	74	92.5
Rat 3	30	11–14	11	25	83.3
Rat 36	75	10–30	11	60	80
Duck 3a	150	9–16	11	144	96

Average percentage of viability over 11 days.....85%

In order to determine the effect of low temperature on metacercariae, a finger bowl containing several hundred larvae encysted on the glass was placed outside on a window sill on March 2, 1938, for 11 days, during which time the temperature varied from 8° Fahrenheit to 45° F. During the night of March 3 the temperature dropped to 8° F. and the water in the dish was frozen solid for at least 15 hours. The dish was then brought into the laboratory, and as the ice melted the cysts came loose from the wall of the dish and fell to the bottom. They were examined several hours after all the ice had melted and were found to contain living metacercariae actively moving within the cyst. About 120 of these metacercariae were fed to rat 31 which was killed 16 days later. Eighty 16-day old worms were recovered from the cecum. The rest of the cysts had been again placed outside, exposed to freezing temperatures almost every night. After 10 days of such exposure, some were fed to rat 33, which, when killed 11 days later, showed only 6 worms in the cecum. The experiments demonstrate that encysted metacercariae of *Z. lunata* are able to withstand complete freezing of the water around them for at least 15 hours and that some are still viable after 10 days of alternate freezing and thawing. This phase of the problem was not carried further because consistently low temperatures were no longer available.

The metacercariae of *Z. lunata* are unable to withstand prolonged drying. A finger bowl containing several hundred viable larvae (30 days old) encysted on the glass was left uncovered without water in the laboratory on March 2, 1938. Twenty-four hours later a few cysts were scraped off and examined and the larvae still looked normal and showed occasional movements. After 48 hours, more were removed and although the metacercariae looked normal no movement was observed. Water was again placed in the dish and metacercariae examined on the next day still showed no movement. One hundred of the cysts were fed to rat 32 which when killed 16 days later contained no worms. Apparently the encysted metacercariae do not survive for long when exposed to the air.

DEVELOPMENT IN THE FINAL HOST.

Observations recorded in the literature on development of amphistomes in the final host are very fragmentary, consisting of only a few scattered and incomplete reports. In the present study, an attempt is made to investigate this stage in the life history rather completely since the experimental hosts, rats and ducks, are favorable for such studies. These hosts show practically a 100 per cent. susceptibility to infection with metacercariae of *Zygocotyle lunata*. All the ducks (13) fed became infected on the first experimental feeding, as did all the rats (more than 60) except those few which were fed metacercariae shown experimentally to be not viable. Both rats and ducks were given the encysted larvae in a few drops of water which was soaked up in small pellets of bread. The pellets were pushed down the throats of the ducks, and the rats after having food withheld for 24 hours ate the bread readily. In some cases the piece of bread was moistened with milk to hasten its ingestion by the rats. Apparently few of the cysts were destroyed in the chewing process, for as described previously, at least 85 per cent. of the metacercariae developed in the experimentally-fed rats (Table 3).

The single sheep (an adult ram) which became infected with *Z. lunata* did not take the infestation on the first feeding. On October 21, 1937, the ram was given 109 viable metacercariae which were mixed with a handful of moistened oats in a porcelain evaporating dish. The cysts stuck to the moist oats and were undoubtedly ingested. Ten fecal examinations were made between November 26, 1937, and March 1, 1938. All were negative. The cysts may have been destroyed in the chewing process. On March 3, 400 metacercariae were placed in a small amount of water in an evaporating dish. Since the ram had been deprived of water for 48 hours he drank the water and swallowed most of the cysts. He was similarly given 600 metacercariae two days later. In three examinations conducted on May 19, 21 and 26, eggs of *Z. lunata* appeared in considerable numbers in the feces. Since the ram was being used for other experiments, no opportunity was afforded to follow the course of the infestation or to obtain the worms. Therefore, this host record rests entirely on the presence of eggs of *Z. lunata* in the feces. However, since thousands of eggs of this species had been collected and studied from the rats and ducks during this period, little, if any, possibility of error in identification of the eggs exists. The only other flatworm to which the ram was exposed was the tapeworm, *Moniezia*. It also carried nematode worms, as evidenced by the presence of nematode eggs in the feces from time to time.

Two pigeons were each fed 50 viable metacercariae on October 23, 1937. Fecal examinations were consistently negative, and, when on

January 29, 1938, both were killed, no worms were found. Similarly a rabbit fed metacercariae showed no eggs of *Z. lunata* in the feces and no worms were found when it was killed 58 days later. No attempts were made to infest other pigeons and rabbits.

In nature, the final host becomes infected with *Z. lunata* by ingesting the encysted larva from pond weeds, or ducks may eat small snails, the shells of which bear encysted metacercariae. In the laboratory, the snails, both naturally and experimentally infested, and other snails which may be in the same dish, become encrusted with many cysts. The 3 cysts shown in Fig. 18 are held together by an underlying piece of thin snail shell on which they were deposited. Similarly some of the uninfected *Helisoma antrosum* collected along with those naturally infected carry a few cysts on their shells.

Excystment probably does not occur in the upper intestine. Rats 22 and 25 were each fed 100 viable encysted larvae on December 30, 1937, and were killed fifteen hours later. In both cases examination of the small intestine yielded no young worms or encysted larvae in that region. In the cecum many newly excysted worms, most of which were not yet attached to the wall of the cecum, were found. Only 30 were collected from each of the hosts, although many more were present. The ceca of both rats contained numerous metacercariae which were still encysted. In rat 22, 25 were collected and these when dissected out from within the softened cyst wall were alive and active. From this observation and the fact that at least 85 per cent. of the metacercariae regularly excyst and develop for at least several days in the final host (Table 3), it seems apparent that excystment usually occurs in the cecum in rats. No evidence on this point is available for ducks.

Zygocotyle lunata occurs normally as a parasite in the cecum of certain water birds, and has been reported from the cecum in the ruminants, *Cervus dichotomus*, by Diesing (1836), and *Bos taurus*, by Price (1928). In the present studies, specimens collected from experimentally infected ducks were always found in the ceca except for one worm in the bursa of a duck killed 14 days after infestation. No specimens were present in the intestine of any of the ducks examined. In the experimental rats the worms were usually found only in the cecum, but in each of 2 cases, 2 worms were found in the large intestine. In agreement with the opinion of Gower (1938), it is believed that the intestine in birds is an abnormal habitat for *Zygocotyle*, and that the parasites merely move out of the cecum after the death of the host. Specimens located in the large intestine of the rat are on the way out of the host in the normal reduction of numbers that occurs. As will be described later in the present paper, the parasites are still intact when extruded with the feces. The worms were usually found within one inch of the distal ends of the ceca in freshly killed ducks.

Table 4.

Experimental infestation of ducks with *Zygocotyle lunata*.

Host No. and date of first feeding	Number of metacercariae fed	Sexually mature, first eggs in feces, (days)	Days elapsed before killing host	Number of worms recovered
1, 10/14/37	50	41	744 10/28/39	0 (Eggs in feces on 10/26/39)
2, 10/14/37	50	—	14	4
3, 10/14/37	50	41	47	7
4, 10/30/37	100	—	14	6
10/14/37	1?	—	31	1
5, 12/13/37	5	—	7	1
1a, 11/20/37	100	44	359	2
2a, 11/20/37	100	44	56	17
3a, 11/20/37	150	—	11	144
4a, 11/20/37	60	—	286	10
5a, 11/20/37	60	—	507	5
6a, 11/20/37	60	—	711	3
12, 7/18/39	100	42	—	—
13, 7/18/39	100	42	—	—

Some variation exists in the time required for *Z. lunata* to develop to sexual maturity. In ducks, they develop more rapidly than in rats. As indicated by the earliest appearance of eggs of *Z. lunata* in the feces, ducks 1 and 3 contained sexually mature worms at 41 days, ducks 12 and 13 at 42 days, and ducks 1a and 2a on the 44th day after experimental infestation (Table 4). A greater amount of variation was observed in the time elapsed before appearance of eggs in the feces of the rat hosts. As shown for 6 of the rats in Table 5, the required time varied from 46 to 61 days. The rate of development is influenced to some extent by the number of worms present. Rat 6, in which the worms developed to maturity most rapidly, harbored only 8 large, mature specimens when killed five days after the first appearance of eggs in the feces. On the other hand, rats 8, 10 and 15, in which from 58 to 61 days were required before eggs appeared in the feces, harbored 23, 30 and 34 specimens respectively, when killed a few days later (Table 5). Rats 8 and 15 contained some specimens which were still not quite mature and contained no eggs in the uterus. The variation is probably related to the large number of worms present, since, as will be shown later, the rate of development is influenced by crowding. When only a few specimens are present, they tend to be all of the same size and at the same stage of development, whereas in hosts harboring many worms, the size and rate of development vary considerably. For example, rat 15, killed 70 days after a single feeding of metacercariae, contained 34 specimens of *Z. lunata* which after fixation varied in length from 3.4 mm. to 5.4 mm. and in width from 1.5 mm. to 1.9 mm., and some were not yet sexually mature although as stated above, eggs were present in the feces ten days earlier, indicating that some were mature at that time.

The faster rate of development of *Z. lunata* to

TABLE 5.

Reduction in number of worms with increase in age of infestation in rats.

Rat No.	Metacercariae ingested by host: Number	Metacercariae ingested by host: Age (days)	Mature, first eggs in feces (days)	Days elapsed before killing host	Number of worms recovered	Percentage of worms recovered
26	150	20–30		22	120	80
4	30	10–14		23	25	83.3
5	30	10–14		45	23	76
6	30	10–14	46	51	8	26
8	70	12–18	58	59	23	32
10	70	12–18	61	61	30	42
15	50	20–40	60	70	34	68
13	50	20–40		92	28	56
14	50	20–40		92	2	4
48	50	138		169	1	2
49	50	138		169	2	4
37	75	10–30		184	2	2.6
38	75	10–30		184	0	0
39	75	10–30		184	3	4
40	75	10–30		184	3	4
41	75	10–30		184	0	0
42	75	10–30		184	3	4
43	75	10–30		184	3	4
16	50	20–40		193	0	0
17	50	20–40		193	0	0
18	50	20–40		193	5	10
19	50	20–40		193	0	0
20	50	20–40		193	0	0
21	50	20–40		193	3	6
60	50	?		226	4	8
52	50	?		238	2	4
58	50	20–30	53	343	2	4
50	50	?		424	1	2
28	100	20–30		711	1	1
62	50	20–30	49			

sexual maturity in ducks is related not only to the higher body temperature of the bird host, but also to the fact that the ducks harbor fewer parasites of this species than do the rats. In infected rats, killed any time up to the 11th day after infestation, most of the worms are still present, but after that a gradual loss of worms occurs (Tables 3, 5). In ducks the results are not uniform with regard to the percentage of worms remaining at different ages of the infestation (Table 4), but this is probably due to the fact that variable numbers of metacercariae reach the ceca of the bird, many being eliminated because of this failure to reach the normal site for development. From the data on number and size of worms present at the time when they reach maturity, specimens of *Z. lunata* are fewer and larger, and they mature more rapidly in the duck than in the rat.

Although large numbers of worms may be present early in an infestation (Table 3), relatively few persist for long after sexual maturity is attained. In 20 of the rats listed in Table 5 the infestation was older than 100 days when the host was killed. Not more than 5 were found in any one rat and the usual number was 2 or 3. In 6 of these cases, rats killed 184 or 193 days after infestation harbored no worms at that time although all had shown eggs of *Z. lunata* in the feces at an earlier date. These 6 rats are the only ones from which all the parasites were eliminated. The number of worms remaining in the host after a long period is not related to the number of metacercariae ingested. In old infestations, the hosts which received only 30 metacercariae harbor just as many worms as those which ingested 100 or more cysts.

In the experimental ducks the parasites also occur in small numbers in the older stages of infestation, from 3 to 5 being found in infestations that are from 300 to 711 days old (Table 4). Records in the literature of the collection of *Z. lunata* from naturally infected bird hosts likewise indicate that this species after reaching maturity occurs in only small numbers. Fischoeder (1903) in a redescription of Natterer's original material from the Vienna Museum, had for comparison 2 specimens from *Cervus dichotomus* and 2 from *Anas moschata*. Stunkard (1916) recovered 8 specimens from a duck, some of which were small and newly matured, and Price (1928) reports the presence of from 1 to 6 specimens in each of 4 species of water birds. Gower (1938), in reporting collections of *Z. lunata* from 7 species of water birds from Michigan, states, "In no case have more than four of these worms been present in any host other than the artifically infected one." He had recovered 46 immature *Zygocotyle* which were 2 weeks old from an artificially infected duck. Price (1928) reported an infestation of *Z. lunata* in a cow, *Bos taurus*, which yielded 30 specimens, but many of the worms were immature. On the basis of a comparison of relative size of acetabulum and other organs with body size, it may be concluded that the infestation in the cow was probably recently acquired and that it could have arisen from a single ingestion of metacercariae.

Little is known regarding the nature of the process of reduction in number of worms in the host. That phase of the problem, involving host-parasite reactions of a local or general nature, aside from the simple effects of crowding, is not included in the present study, but the evidence for some mechanism of host resistance is certainly apparent. Stoll (1929), working with the nematode *Haemonchus contortus*, was the first to show that following the acquirement of an initial infestation, a "self-cure" occurred, accompanied by a high degree of protection from further infection. He suggested that other helminthic infections might show a similar host response. The evidence for "self-cure" and resistance is presented for *Zygocotyle lunata* in the present studies.

Immunity. An established infestation with *Zygocotyle lunata* in ducks and in rats prevents a superinfestation with this species. Eight attempts were made to superimpose a second infestation. Table 6 shows the results of experiments involving 5 ducks and 3 rats. Following a single experimental feeding with metacercariae of *Z. lunata*, a second feeding of 50 to 150 metacercariae was given after 6 to 261 days of duration of the first infestation. The hosts were then sacrificed

after periods varying from 4 to 28 days after the second feeding. With the exception of an accidental infestation of one worm in duck 4, no worms were found from the second experimental feedings. In each case, as seen in Table 6, parasites from the second feeding would be easily identifiable and distinctly different in size from the worms actually found, all of which were from the initial experimental feeding. The number of specimens recovered in the various hosts in these planned experiments varied from 2 to 144. It may be concluded that as few as 2 mature worms of this species will make the host immune to further infestation. Further, the experiment on duck 3a shows that an infestation of 6 days duration will prevent a superinfestation, and the data on duck 4a indicate that 10 worms 261 days old will do the same. No extensive information is available as to the minimum number of worms of a given age which will be necessary to produce resistance to further infestation, but the data in Table 6 seem to indicate that a few worms of almost any age will probably be effective.

Table 6.

Data showing immunity to superinfestation with *Zygocotyle lunata*.

Host	First experimental feeding	Days elapsed before second feeding	Host killed		Worms recovered		Worms from second feeding
			Days after 2nd feeding	Days after 1st feeding	Age (Days)	No.	
Duck 3	10/14/37	19	28	47	47	7	0
Duck 4	10/30/37	9	5	14	14	6	0
Duck 4	10/14/37*	17	14	31	31	1	6*
Rat 8	11/15/37	45	14	59	59	23	0
Duck 3a	11/20/37	6	5	11	11	144	0
Duck 2a	11/20/37	52	4	56	56	17	0
Rat 14	11/23/37	69	23	92	92	2	0
Rat 6	10/14/37	41	10	51	51	8	0
Duck 4a	11/20/37	261	25	286	286	10	0

* Accidental infestation with 1 worm (See text).

No attempt was made to determine whether a single worm from the feeding of a single metacercaria would induce the characteristic immunity, but an accidental infection of a duck may throw some light on the probable result. Duck 4, when killed and examined, was expected to have only 14-day old worms, but it contained 6 of these and 1 worm which was obviously much older. Inspection of the data on this duck showed that it had been housed for part of a day, 31 days before, with 3 other ducks (no. 1, 2, and 3) which had just been given an experimental feeding with metacercariae of *Z. lunata*. Comparison of the single large worm obtained with other worms of known age definitely establishes it as being 31 days old. Apparently this duck (no. 4), although not purposely fed with the other 3 ducks, had accidentally picked up a cyst somewhere in the enclosure, possibly from the bill of one of the other ducks which had been fed by dropping cysts with water into their throats from a medicine dropper. This method was later abandoned in favor of placing the cysts in a pellet of bread, as described previously. Duck 4 was fed metacercariae 17 days later and then killed after 14 days, yielding the 6 worms of the 14-day age and one 31 days old. Four were in one caecum and 3 in the other. The results would seem to indicate that a single young worm, 17 days old, is not sufficient to produce resistance to superinfestation. This was the only case in which worms were recovered from more than a single feeding of metacercariae. In all other cases the worms obtained were of approximately the same size, any differences being easily within the range of individual variation in growth rate.

The metacercariae of a second feeding are released from their cysts and may remain in the host for as long as 4 days. Rat 8, given a second feeding 45 days after initial infestation with *Z. lunata*, was examined for eggs in the feces 4 days after the second feeding and small worms were recovered from the feces. Three were found in one medicine dropper of material from the bottom of a settling glass. They were from the second feeding of 4 days before but had not developed to a 4-day stage in growth. They were dead when recovered but their organization was still intact, and when stained and mounted they were found to have progressed to a 2-day stage of development. The measurements for them are included in Table 7 which compares body size, and the sizes of acetabulum and oral sucker in metacercariae dissected from cysts and in worms of from 15 hours to 7 days of development. The 3 worms recovered from the feces of rat 8 agree closely in measurements with those of 2-day-old worms from rat 7. Apparently the reaction of immunity against superinfestation begins to be effective almost immediately after excystment of the metacercariae, since the young worms were extruded at a 2-day stage of development on the 4th day following ingestion of the metacercariae. No 4-day-old worms were found in duck 2a which was killed 4 days after a second feeding with metacercariae.

The mechanism for this resistance to superinfestation with a metazoan parasite such as *Zygocotyle* is not entirely clear. The problem has been investigated by Taliaferro (1940), Chandler (1937, 1939) and others. For the literature and a general review of the mechanism of immunity to metazoan parasites, the reader is

referred to a paper by Taliaferro (1940) in which he states, "Various investigators, including the speaker (16), have stressed the fact that the immunological mechanisms, both humoral and cellular, operative against the larger parasites are identical with those operative against other infectious and antigenic non-infectious foreign materials."

2 worms from the latter host were very much larger, averaging 7.0 mm. in length and 3.2 mm. in width as compared with the average measurements of 5.0 mm. × 2.3 mm. for the more numerous worms in rat 13. Inspection of the range in size between these two groups of parasites demonstrates strikingly the effects of crowding. A similar comparison of the data in

Table 7.

Comparative average measurements of specimens of *Zygocotyle lunata* of ages up to 7 days.

Age of specimen	Length in mm.	Width in mm.	Acetabulum Length in mm.	Acetabulum Width in mm.	Oral Sucker Length in mm.	Oral Sucker Width in mm.	No. of worms measured
Metacercariae dissected from cysts	.495	.201	.171	.165	.063	.059	10
15 hrs. in Rat 22	.516	.198	.174	.164	.082	.082	10
2 days in Rat 7	.772	.260	.243	.230	.099	.105	10
4 days, from feces of Rat 8	.850	.204	.244	.211	.099	.112	3
3 days in Rat 11	.792	.306	.248	.273	.122	.106	10
5 days in Rat 1	.940	.460	.420	.400	.165	.132	10
7 days in Rat 6a	1.200	.590	.462	.442	.211	.165	10

Table 8.

Showing the relation between age, number and size of *Zygocotyle lunata* in rats and ducks.

Days after ingestion of meta-cercariae	Host	No. of worms present	No. of worms measured	Average length in mm.	Average width in mm.	Range in length in mm.	Range in width in mm.	Remarks
17	Rat 31	80	10	2.0	0.86	1.6–2.4	0.6–1.2	Immature
22	Rat 26	120	10	2.47	0.86	2.2–3.3	0.8–1.0	Immature
23	Rat 4	25	10	2.6	1.1	2.5–3.0	1.0–1.4	Immature
31	Duck 4	1	1	4.1	1.5	4.1	1.5	Immature
45	Rat 5	23	10	3.8	1.7	3.1–4.5	1.4–2.3	Some mature
47	Duck 3	7	7	6.1	3.0	5.8–6.5	2.8–3.1	All mature
51	Rat 6	8	8	5.4	2.7	4.7–6.2	2.5–2.9	All mature
56	Duck 2a	17	10	7.9	2.9	7.1–8.7	2.7–3.0	All mature
59	Rat 8	23	10	3.9	1.7	3.4–4.6	1.5–2.0	Some mature
70	Rat 15	34	10	4.1	1.7	3.4–5.4	1.5–1.9	All mature
92	Rat 13	28	10	5.0	2.3	4.2–5.8	1.7–2.7	All mature
92	Rat 14	2	2	7.0	3.2	6.6–7.5	3.1–3.3	All mature
193	Rat 18	5	4	8.2	3.0	7.0–9.5	2.8–3.3	All mature
193	Rat 21	3	3	8.6	3.1	7.0–9.5	3.0–3.2	All mature
226	Rat 60	4	4	6.0	3.1	5.5–6.4	2.7–3.4	All mature
238	Rat 52	2	2	7.3	2.9	6.9–7.7	2.5–3.3	All mature
286	Duck 4a	10	10	6.9	3.54	6.4–7.4	3.2–4.0	All mature
359	Duck 1a	2	2	7.2	3.75	7.0–7.2	3.5–4.0	All mature
424	Rat 50	1	1	10.5	3.5	10.5	3.5	All mature
507	Duck 5a	5	5	7.4	3.78	6.5–8.0	3.5–4.2	All mature
711	Rat 28	1	1	8.5	4.0	8.5	4.0	All mature
711	Duck 6a	3	3	9.1	4.65	9.0–9.2	4.6–4.7	All mature

Size and Longevity of Z. lunata. As pointed out previously, the size of worms of any given age as well as the rate of development to sexual maturity varies with the number of worms in the host. When many worms of the same age are present, the size varies more and the worms are smaller than when only a few worms of the same age are present. Table 8 shows the average size, range of size, and the number of worms present at various ages from 17 days to 711 days in rat and duck hosts. The relationship between number and size of specimens is illustrated well by the data on rats 13 and 14, both of which on the same day were fed equal numbers of metacercariae from the same snail host. Rat 13 harbored 28 worms in the cecum and rat 14 only 2. The Table 8 for rats 6 and 8 killed 51 and 59 days after experimental infestation and yielding 8 and 23 worms respectively shows the same relationship. On the other hand worms of the same age occurring in approximately equal numbers in different hosts tend to be more uniform in size. It was observed in ducks that if one cecum contained only one worm and the other harbored several, the single worm which occupied a cecum by itself was considerably larger (8.04 mm. × 4.25 mm.) than the other worms averaging 7.2 mm. × 3.6 mm. from the other cecum (Duck 5a).

Rankin (1937) also pointed to the relationship between number and size of trematodes within a host and suggested that crowding may be the factor concerned. He stated, "It has been ob-

served also, that the trematodes *Brachycoelium*, *Plagitura*, and *Megalodiscus*, when present in large numbers, are usually small, though mature. Crowding of many individuals within a small area may account for small size, for when these flukes occur in small numbers, they are much larger."

The smallest sexually mature *Zygocotyle lunata* obtained from experimentally infected hosts was 3.1 mm. in length and 1.4 mm. in width, and the largest specimen measured 9.2 mm. × 4.7 mm. The smaller worms were collected from a 45-day infestation in rat 5. From the smaller size the worms continue to grow regularly as they get older; and, as seen in Table 8, rat 50 contained a 424-day-old worm measuring 10.5 mm. × 3.5 mm. The worms obtained after 711 days of growth in rat 28 and in duck 6a are still larger, the largest of the three from the duck being 9.2 mm. in length and 4.7 mm. in width. This represents an increase of many times in volume over the size of the worms when first sexually mature. These worms are larger than any of this species reported heretofore in the literature.

The sizes recorded in Table 8 are comparable since the same killing procedure was followed in all cases. The worms were slightly flattened on a large 2 × 3 inch glass slide under the weight of an ordinary 1 × 3 inch slide and killed with an aqueous saturated solution of corrosive sublimate containing 3% of acetic acid. The Table demonstrates that worms when first mature are larger in ducks than they are at a similar stage of development in rats. Ducks 3 and 2a contained mature worms 47 and 56 days old respectively which are much larger than the newly matured worms from rats 8 and 15 in which the parasites are 59 and 70 days old respectively. Some of the disparity in size is due to the fact that a greater number of worms were present in the rats. In the older worms the difference in size lessens until it disappears altogether in those of 200 days or older from the two host species. In these cases worms of the same age tend to be of the same size regardless of the host in which they developed.

Specimens of *Zygocotyle lunata* may live for more than 2 years in ducks and rats. In the rat, the length of life of *Z. lunata* is to some extent limited by the life span of the rat. Rat 28 was about 3 months old when fed metacercariae on January 31, 1938. Eggs of *Z. lunata* were collected continuously from the feces and an examination made on January 5, 1940, yielded numerous eggs. By this time, the rat, a male, was becoming quite feeble and slept most of the time. On January 12, 1940, this rat, barely able to walk across the cage, was killed and one large worm was recovered from the cecum 711 days after ingestion of the metacercariae. The rest of the intestine was not examined. The worm was very active and contained many eggs in the uterus. It extruded at least 40 normal-appearing eggs in the dish of water before it was killed and fixed. These eggs were washed and they developed normally, indicating that the old worms are still able to reproduce and would probably live much longer. Duck 6a was also killed 711 days after a single experimental feeding with metacercariae and the 3 worms recovered were the same as the one of the same age from the rat, except that they were slightly larger.

Another duck (no. 1) was very disappointing as a subject for longevity records on *Zygocotyle*. It was given a single feeding of encysted larvae on October 14, 1937 (Table 4), and showed eggs of *Zygocotyle* in the feces after 41 days and intermittently from that time on. After about 18 months of infestation, only a few eggs were obtained in each positive fecal examination but some were collected on October 20 and 26, 1939. Two days later, October 28, the duck was killed but no worms were found. Apparently the infestation had been lost during the preceding 48 hours. The last eggs had been collected 742 days after infestation and on the 744th day the worms were gone. This does not necessarily indicate, however, that the worms live for only about 2 years.

In *Z. lunata*, the parasites apparently keep on increasing in size as long as they live, at least up to 2 years. It has been shown that many, and in a few cases all, the worms are lost long before this age is attained. Natural infestations in ducks probably carry over from one year to the next just as the experimental infestations in laboratory-raised ducks do. Gower (1938a), in a study of seasonal abundance of the parasites of wild ducks, found *Zygocotyle* in 12 of 104 ducks examined, with a slightly higher percentage of infestation during the summer and a sudden drop in the autumn. In view of the present data regarding longevity of metacercariae and of adults of *Zygocotyle*, the significance of Gower's data on seasonal abundance in this species seems doubtful. The variation reported in numbers of ducks infected at the different seasons: 17.5% in spring, 23% in summer, 4% in fall and 10% in the winter, is probably not significant. The birds may become infected with *Z. lunata* at any season but probably pick up the cysts less readily in winter when ice serves to lessen the chances for infestation. However, with the relatively long span of life of the parasite, very little difference probably occurs in the incidence of this form at different seasons.

Measurements of *Z. lunata* illustrate the need for caution in attaching much importance to size for specific diagnosis. As in some other trematodes, extreme variation exists in size of adult specimens. In the experimentally raised and genetically similar material dealt with in the present paper, old mature worms are many times larger in volume than younger, newly-matured individuals (Table 8). Measurements for the earlier stages of development are presented in Table 7. Body growth and the growth in size of organs proceed proportionately in a regular progressive manner up to the time of sexual maturity. Miller (1939) reported a decrease in size of developing *Postharmostomum laruei* in mice during the first 30 hours, and suggested

that a similar decrease probably occurs in other digenetic flukes upon entering the bodies of their definitive hosts. It is obvious from Table 7 that no such decrease occurs in *Zygocotyle*. The specimens after 15 hours in the rat are slightly larger than metacercariae, even though they have emerged from their cysts only a short time before. Some metacercariae have not yet excysted after 15 hours.

Table 9 presents the comparative measurements of worms varying in age from 11 to 711 days. The measurements are averages of several worms in most cases and are taken from similarly stained and cleared whole mounts. It is apparent that size of body and size of the contained organs increases proportionately up to sexual maturity. After sexual maturity is attained, however, the body organs do not continue to increase in size significantly in proportion to the increase in gross size of the worm as it becomes older and larger. Some individual variation occurs, but the apparent slight increase in size of some organs, as the acetabulum, does not parallel the rate of increase shown in total body measurements (Figs. 20–25). A comparison of body size and organ size in specimens 47 and 51 days old with those of specimens 92 to 711 days old (Table 9) shows little significant growth in size of organs but a very considerable increase in gross size.

Table 9.

Comparative measurements in mm. of stained and cleared specimens of *Zygocotyle lunata* at different ages. (Averages of several specimens in most cases.)

Age in days and host	Length	Width	Acetabulum	Oral sucker	Oral evaginations	Esophageal bulb	Anterior testis	Posterior testis	Ovary
11—Rat 3	2.14	.825	.594 × .548	.264 × .264	.099 × .066	.118 × .092	.118 × .079	.115 × .069	.060 × .046
14—Duck 4	1.87	.740	.745 × .567	.258 × .258	.085 × .070	.145 × .090	.099 × .066	.099 × .066	.055 × .050
23—Rat 4	2.60	1.12	1.06 × .878	.396 × .376	.175 × .112	.231 × .151	.455 × .231	.429 × .264	.132 × .132
31—Duck 4	4.10	1.48	1.49 × 1.06	.429 × .396	.198 × .112	.265 × .178	.330 × .198	.297 × .198	.264 × .158
45—Rat 5 (Immature)	3.33	1.28	1.06 × .807	.488 × .475	.198 × .118	.218 × .132	.396 × .297	.396 × .363	.119 × .107
45—Rat 5 (Mature)	3.10	1.70	1.05 × .825	.462 × .430	.180 × .127	.264 × .190	.660 × .264	.660 × .297	.198 × .132
47—Duck 3	6.10	3.00	1.59 × 1.06	.521 × .462	.205 × .129	.328 × .219	1.09 × .512	.972 × .549	.476 × .264
51—Rat 6	5.48	2.65	1.30 × 1.06	.613 × .628	.264 × .158	.383 × .208	1.04 × .552	1.04 × .594	.445 × .255
92—Rat 14	7.05	3.20	1.48 × 1.19	.552 × .637	.212 × .170	.382 × .255	.892 × .425	.935 × .595	.425 × .297
193—Rat 21	8.23	2.97	1.59 × 1.27	.689 × .637	.243 × .212	.403 × .218	1.10 × .743	1.18 × .743	.637 × .254
359—Duck 1a	7.20	3.75	1.48 × 1.48	.552 × .552	.212 × .170	.382 × .245	1.06 × .425	1.06 × .425	.637 × .255
507—Duck 5a	7.71	4.07	1.82 × 1.48	.595 × .595	.212 × .191	.362 × .232	1.44 × .595	1.57 × .637	.722 × .275
711—Duck 6a	9.10	4.65	1.91 × 1.48	.660 × .627	.212 × .170	.380 × .255	1.10 × .510	1.06 × .552	.637 × .264

Miller (1939) made observations on the rate of growth in *Postharmostomum* and described an increase in both length and width between worms which are 400 hours old, measuring 3.07 mm. by 1.09 mm., and worms 1,100 hours old which are 3.99 mm. long and 1.32 mm. in width. The worms are apparently sexually mature at 400 hours. He presented no data on size for ages intermediate between 400 and 1,100 hours, and did not report the range of variation among the specimens. The average measurements showing an increase in length and width of the worms during the stages preceding sexual maturity (before 400 hours) are not significant with respect to specific diagnosis since trematodes commonly undergo a growth period of this sort prior to maturity. On the basis of the increase in size following sexual maturity, he emphasized the undesirability of placing too much importance on size in specific diagnosis. With reference to his measurements, he says, "The differences in size between different ages of the same fluke, as shown here, are comparable to those which have been used, at times, as a basis for indicating different species." While this may be true, it should be pointed out that the reported increases in length of 30 per cent., and in width of 17 per cent., following sexual maturity in *Postharmostomum*, are not greater than the variation in size which may occur in different specimens of *Zygocotyle* of the same age collected from a single host animal. In *Zygocotyle*, the 70-day-old worms collected from rat 15 (Table 8) varied in length from 3.4 mm. to 5.4 mm., the longer specimens being 58 per cent. greater in length than the smaller ones. Similarly the 92 day old specimens from rat 13 exhibit a 38 per cent. difference. As shown earlier, a wider variation in size of worms occurs in the hosts which harbor a large number of specimens than in those which contain relatively few. In *Zygocotyle*, worms of a given age are larger when only a few are present than when many have developed together in the same host. The possibility exists that this relationship may be a factor in the size difference between 400 and 1,100 hour worms in *Postharmostomum*, since 28 flukes of the 400 hour group were present in the host animal, while only 5 were found in the mouse which yielded the larger 1,100 hour worms. In view of the relationships between number, size and age of specimens demonstrated in *Zygocotyle*, the data given by Miller for *Postharmostomum laruei* do not justify the conclusions presented by him regarding trematodes in general.

During the course of the infestations with *Zygocotyle*, frequent fecal examinations for eggs of the parasite were conducted. Interesting

differences appear in the results obtained from feces of rats and ducks. The same procedure was followed in all cases. Fecal samples were broken up in water and strained through a fine wire screen into a settling dish in which the material was then washed several times by the decanting method. The eggs were collected under a binocular dissecting microscope. In every fecal examination of rats infected with mature *Zygocotyle*, eggs were obtained. Rat 28 was examined more than 50 times over a period approaching two years. The number of eggs collected from 8 fecal pellets varied from 650 in the early months of the infestation to as few as 25 during the last few weeks of the life of the rat. Some were always present. Feces of infected ducks, however, did not always contain eggs. For some reason not clear to the writer, a bird would apparently be negative, and then a week or two later, eggs would again be present. Daily fecal examinations sometimes failed to yield eggs for periods up to 2 weeks, after which eggs would be plentiful again for a similar period in daily collections of feces. Of 78 recorded examinations of fecal samples from duck no. 1, 43 were positive and 35 were negative, but the significant feature is that the feces were often consistently negative or positive for periods of from 8 to 13 successive days. Although a periodicity in egg production for *Zygocotyle* in ducks is suggested by such data, it apparently does not occur since it certainly does not exist in the rat host. The results cannot be accounted for by loss of worms and subsequent reinfestation, because no opportunity was afforded for reinfestation. The only explanation that can be offered at present is the element of chance combined with the peculiar action of the ceca in ducks. Fecal samples from other infected ducks were similarly negative from time to time for *Zygocotyle* eggs, although the hosts were unquestionably infected. The data are not sufficiently complete to draw any conclusions other than that failure to find eggs of trematodes living in the ceca of ducks, after only a few fecal examinations, does not necessarily indicate that the bird is not infected. Caution must be exercised in using such ducks for feeding experiments in life history studies.

The Adult.

The morphology of *Zygocotyle lunata* has been adequately described by Fischoeder (1903), Stunkard (1917), Price (1928) and Gower (1938). Since the tables presented in the present work include comparative measurements and other information concerning variation within the species, no detailed description of the adult is given. Figures 20–25 are photomicrographs of whole mounts at various stages of development, from 5 days to 711 days old, showing the relation between size of organs and size of the body. Figures 23 and 24 illustrate, among other things, the fact that the acetabulum does not increase in size in proportion with increase in size of body as the worms grow older and larger, but is proportionately smaller in older than in younger worms. The actual sizes and ages of these specimens is given in the explanations of the figures and the average sizes for the organs appear in Table 9.

Stunkard (1917) transferred *Amphistoma lunatum* to the genus *Zygocotyle* and erected a new subfamily, with *Zygocotyle* as type genus, to contain it. The subfamily Zygocotylinae was first named by Ward (1917). Bhalerao (1931) described a new genus and species, *Stunkardia dilymphosa*, and placed it with *Zygocotyle* in the subfamily Zygocotylinae. On the basis of the structure of *Stunkardia dilymphosa*, Bhalerao found it necessary to modify the definition of the subfamily. He described two pairs of lymph vessels in the new species, as indicated by the specific name, and therefore, in the new diagnosis he includes, "Lymphatic system with a pair of dorsal and a pair of ventral lymph canals." The lymph system of *Zygocotyle*, the type genus, had not been described at that time. Willey (1933) showed that *Zygocotyle* possesses only a single pair of main lymph channels, and pointed out that, "The citation with respect to the lymph system in the subfamily must be modified or else eliminated entirely from the definition." Furthermore, in view of the report by Price (1928), showing *Bos taurus* as a host of *Zygocotyle*, and on the basis of the experimental infestations obtained in a sheep and in rats by the present author, that part of Bhalerao's diagnosis concerning the host relationships of the subfamily Zygocotylinae, "Parasites of birds and reptiles," must therefore be changed to include parasites of mammals. Although Näsmark (1937) cited the work of Price (1928), showing that *Zygocotyle* occurs in the cow, supporting the earlier host record by Diesing (1836) of this species from a deer, he apparently did not accept it as valid. He stated (p. 436), "Hitherto known definitely only from ducks in America. A specimen from *Bos taurus* may be wrongly labelled (?)." The experimental infections in the rat and sheep accomplished in the present work certainly leave no further doubt that *Zygocotyle* occurs in mammals as well as in birds. In his diagnosis of the subfamily Zygocotylinae, Näsmark (1937) stated (p. 444), "So far as is known with certainty they parasite (sic) only in American ducks." Nevertheless he cited the work of Bhalerao (1931) who placed *Stunkardia dilymphosa*, from a reptile, in the subfamily Zygocotylinae. It is not clear whether Näsmark inferred that *S. dilymphosa* does not belong in the subfamily or whether the statement was made in error. If *S. dilymphosa* should not be included, as seems likely to the present writer, then *Zygocotyle lunata* is the only species as yet known in the subfamily Zygocotylinae.

Summary.

Beginning with cercariae (*C. poconensis* Willey, 1930) from naturally infected snails of the species *Helisoma antrosum*, the complete life cycle of *Zygocotyle lunata* was demonstrated in the laboratory. The cercariae encyst in the open

and metacercariae fed to laboratory-raised rats, ducks and a sheep developed into adult worms. Eggs collected from feces were embryonated and the miracidia penetrated laboratory-raised *Helisoma antrosum* from which cercariae emerged within from 32 to 49 days after exposure. The morphology and activities of the miracidia are described. In the snail the miracidium metamorphoses into a sporocyst which produces rediae. Some of the rediae contain a single daughter redia at the anterior end as well as cercariae developing at the same time in the same larva. Other rediae contain only young cercariae. It is probable that each redia produces one daughter redia and proceeds from that time on to produce cercariae only. This explains the presence of rediae of all sizes during all stages of the infestation which persists for more than 10 months in the snail. The sporocyst becomes exhausted and disappears soon after the first cercariae are shed.

Cercariae leave the redia while still immature and continue their development in the lymph spaces of the snail, after which they emerge and encyst in the open. Encysted metacercariae are infective immediately and some are still infective when fed to rats after 138 days in the laboratory at room temperature. They will withstand freezing temperatures but are not viable if left out of water for 48 hours at room temperature. No development occurs in the cyst.

In the rat the metacercariae excyst in the cecum and develop to maturity. They are normally parasites of the cecum and have been reported from the ceca of various water birds, a deer and a cow. The present experimental infestations in the rat and sheep constitute new host records for *Zygocotyle*. Sexual maturity of *Z. lunata* is attained faster in ducks (41–44 days) than in rats (46–61 days), and the rate of development is influenced by the number of worms present, more time being required when many worms are present. As the age of the infestation increases, the number of worms is reduced and only a few persist for long after sexual maturity is reached. The host usually eliminates most of them within the first 3 months and retains only 1 to 5 thereafter. An established infestation of *Z. lunata* in rats and ducks prevents a superinfestation with this species.

Size of worms varies with the number present. When many worms of the same age are present, the size varies more and the flukes are smaller than when only a few of the same age are developing together. The data demonstrate the effects of crowding. *Z. lunata* may live for more than 2 years in rats and ducks, and following sexual maturity the worms continue to increase in size. The smallest sexually mature worm obtained experimentally was 3.1 × 1.4 mm. and the largest measured 9.2 × 4.7 mm. when 711 days old. Although gross size increases greatly, the sizes of organs in the body do not increase proportionately. The value of size as a species difference in trematodes is discussed. Regular fecal examinations of ducks infected with *Z. lunata* often failed to show eggs of the parasites which were resident in the ceca. Feces of infected rats always yielded eggs. Ducks should not be considered negative for worms living in the ceca after only a few negative fecal examinations.

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EXPLANATION OF THE PLATES.

Plate I.

Photomicrographs of eggs and miracidia of *Zygocotyle lunata.*

Fig. 1. Egg, living, from feces. Length, 0.145 mm.
Fig. 2. Eggs, showing variation in shape and size.
Fig. 3. Embryo after 13 days of development.
Fig. 4. Embryo after 18 days of development.
Fig. 5. Embryo after 21 days of development.
Fig. 6. Miracidium, living, slightly flattened under a coverglass. Length, 0.210 mm.
Fig. 7. Miracidium, whole mount of silver impregnated specimen, showing entire thickness. Length, 0.191 mm.
Fig. 8. The same miracidium as in figure 7, with higher initial magnification showing critical focus on the upper surface. Length, 0.191 mm.

Plate II.

Free-hand drawings of some larval stages of *Zygocotyle lunata.*

Abbreviations.

ap, apical papilla
br, dorsal nerve mass
ce, cercaria
ci, cilia
cn, epidermal cell nucleus
dr, daughter redia
ed, excretory collecting duct
ep, excretory pore
es, eye-spots
fl, flame cell
gb, germ ball
gc, germ cell
gn, 'gut' nucleus
in, intestine
pg, 'primitive gut'
ph, pharynx
re, redia
sc, subepithelial cell nucleus
sp, aperature for sensory papilla
T1, Epidermal cell of 1st tier
T2, Epidermal cell of 2nd tier
T3, Epidermal cell of 3rd tier
T4, Epidermal cell of 4th tier
ve, vesicle

Fig. 9. Miracidium from dorsal aspect; reconstruction from observations on numerous living and fixed specimens. Average length, 0.195 mm.
Fig. 10. Reconstruction of sporocyst from sections of experimentally infected *Helisoma antrosum,* 28 days after penetration of the miracidium. Length, 0.305 mm.
Fig. 11. Young redia containing single, large daughter redia, from living specimen. Length, 0.330 mm.
Fig. 12. Older redia containing single young daughter redia and several developing cercariae; 25 days after penetration of the miracidium, living specimen. Length, 0.860 mm.
Fig. 13. Redia, containing active, older daughter redia which is ready to emerge, and several developing cercariae; from living specimen in snail crushed 47 days after penetration of the miracidium. Length, 0.792 mm.

Plate III.

Photomicrographs of various stages in the life history of *Zygocotyle lunata.*

Fig. 14. Section of sporocyst reconstructed in fig. 10. Note pharynges of 2 rediae and portions of intestine of 2 others.
Fig. 15. Redia, living, containing developing cercariae and germ balls. Length, 0.775 mm.
Fig. 16. Ventral aspect of mature cercaria, living, under medium pressure of coverglass. The tail is still attached but out of focus. Note branched excretory collecting ducts as outlined by excretory concretions. Length, 0.990 mm.
Fig. 17. Whole mount of fixed and stained cercaria from ventral side, killed under slight pressure. Length, 0.660 mm.
Fig. 18. Encysted metacercariae attached to the surface of the shell of a snail. Average diameter, 0.289 mm.
Fig. 19. Ventral aspect of whole mount of a 10-day old metacercaria dissected from its cyst. Length of specimen, fixed and stained, 0.489 mm.
Fig. 20. Ventral aspect of young immature adult after 5 days of development in rat 1. Length, 0.924 mm.

Plate IV.

Photomicrographs of whole mounts of fixed and stained specimens of *Zygocotyle lunata* at various stages of development in the final host.

Fig. 21. Ventral aspect, after 11 days in rat 3. Length, 2.21 mm.
Fig. 22. Ventral aspect, after 14 days in duck 4. Length, 2.12 mm.
Fig. 23. Ventral aspect, after 23 days in rat 4. Length, 3.04 mm.
Fig. 24. Ventral aspect, after 51 days in rat 6. Length, 6.07 mm.
Fig. 25. Dorsal aspect, after 711 days in duck 6a, photographed by reflected light. Length, 9.0 mm.